Bacterial Activity Associated with the Decomposition of Woody Substrates in a Stream Sediment

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Ground bark and heartwood from Alnus rubra and Pseudotsuga menziesii were added to a muddy sediment from a small Oregon stream and incubated in situ. Carbon dioxide and methane production rates were increased by all amendments, the biggest increase being shown with A. rubra wood. Except for sediment amended with A. rubra wood, nitrogen fixation rates from all treatments (including the control) were approximately 0.1 nmol/g per h throughout the 6-month study period. Contrary to expectations, neither bark had a noticeable adverse effect on microbial activity, but the A. rubra wood promoted nitrogen fixation. These results help to explain the faster rate of decomposition of A. rubra wood in water compared with that of P. menziesii described in the literature. The uptake kinetics of glucose (V_max) did not follow the same pattern as gas evolution.

Streams and rivers in forested regions contain large quantities of wood in the form of twigs, branches, tree trunks, and fine particles; amounts of up to 80 and 25 to 40 kg/m^2 have been recorded in the redwoods and in western Oregon streams, respectively (19). Small streams have more wood per unit area than large rivers because they do not have the power to flush out large bole wood. Wood debris becomes fragmented and deposited in the sediments through physical abrasion, microbial activity, and the activities of the lotic invertebrate fauna (2). This paper is concerned with the response of the sediment microflora in a small stream to the addition of four woody substrates. The principal microbial activities monitored were the production of carbon dioxide and methane, anaerobic nitrogen fixation, and glucose uptake kinetics (22). The substrates used were the heartwood of Pseudotsuga menziesii (Douglas fir) and Alnus rubra (red alder) and bark from the same trees. Both species are common riparian trees in western Oregon, but A. rubra wood is known to decompose in streams much faster than P. menziesii wood (2). In addition, P. menziesii is a major timber and plywood species in the United States, and hence much is known of the chemistry of its wood and bark. Bark was used not only because, like wood, it is a natural allochthonous material, but also because its high tannin content (especially in P. menziesii) might be expected to influence its rate of utilization.

MATERIALS AND METHODS

Site description. Experiments were carried out from January to August 1981 in Berry Creek, a second-order forest stream approximately 16 km north of Corvallis, Oreg. The riparian vegetation consisted of a mixed woodland (including A. rubra and P. menziesii) which formed a more or less closed canopy over the water. Discharge was regulated by a control valve and relief channel such that the flow through the experimental section did not exceed 0.03 m^3/s. The mean standing crop of wood (excluding fine particles) in Berry Creek has been estimated at 2.73 kg/m^2 (2), but not all of this material was buried in the sediment. Also, the regulation of the discharge prevents winter spates which would otherwise wash some of the woody matter out of the system. The temperature, pH, and specific conducance of Berry Creek vary from approximately 4°C, pH 7.3, and 86 μS in the winter to approximately 15°C, pH 7.7, and 132 μS in the summer. Further information on the stream's characteristics can be found in the detailed description of Warren et al. (20).

Collection and preparation of substrates and sediment. The substrates were collected from a single specimen of each tree species which had fallen naturally into a stream. It is not known when the selected trees had fallen, but they were in contact with the flowing water and the bark was still present although easily detached. Pieces of bark and heartwood were removed to the laboratory, reduced to a manageable size, and dried at ~ 70°C. Higher temperatures cause the loss of terpenes and other volatile constituents. Each substrate was then ground through a Wiley mill with a 20-mesh screen.

Thick organic mud was obtained from the bottom of a pool in Berry Creek and sieved on site to remove

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coarse debris and the larger invertebrates. Sieves of mesh size 25, 19, 13, and 10 mm were used consecutively. The mud was then taken to the laboratory, and portions were thoroughly mixed with each substrate separately at a concentration of 2% (wt/vol). Plastic pots (approximately 10 cm in diameter) were then filled to the brim with either a sediment-substrate mixture or unamended mud. Each pot held roughly 400 ml (i.e., about 85 g [dry weight]), and there were 15 replicates of each treatment. The pots of mud were returned to Berry Creek and attached to small stakes, and the stakes were inserted into the same sediment bed from which the mud was taken such that the rims of the pots were more or less level with the surface of the sediment. The period from mud collection to pot placement was about 24 h. Individual pots were then removed as required at intervals up to 28 weeks after deposition, and analyses were begun within 1 h of removal from the stream.

**Measurement of microbiological variables.** Nitrogen fixation was estimated by the acetylene reduction technique, during which ethylene gas is produced. This ethylene, along with the carbon dioxide and methane, was determined on a Hewlett-Packard 5840 gas chromatograph. Paired Porapak R columns (2 m long) were used for CO₂ separation in association with a thermal conductivity detector at an oven temperature of 35°C and with helium carrier gas (flow rate, 24 ml/min). A flame ionization detector was used for methane and ethylene with nitrogen as the carrier gas (flow rate, 15 ml/min). Injector and detector temperatures were 150 and 250°C, respectively, for all analyses.

Gases (0.3 ml) for analysis were taken by syringe from the headspace above 3-ml cores of mud contained in 8-ml glass tubes closed with butyl rubber stoppers. The 3-ml cores were taken vertically from the sample pots with a 5-ml plastic syringe with the end removed. Carbon dioxide production rates were determined from tubes incubated aerobically. For nitrogen fixation and methanogenesis determinations, cores were taken in a glove bag filled with oxygen-free nitrogen, and the same gas was used to purge the tubes after they had been stoppered. All cores were incubated in the dark for 72 h at the prevailing stream sediment temperature. For nitrogen fixation 1 ml of the headspace was replaced by acetylene generated from calcium carbide, and these tubes were also used as the methane controls (11). For all gases four separate concentration estimations for each tube were made at approximately 24-h intervals, and production rates were calculated by linear regression after appropriate corrections for volume reduction due to the sampling procedure. Triplicate cores were taken for each gas on each occasion.

Kinetic uptake studies were performed on 1-ml samples from each pot diluted with 1 liter of sterilized Berry Creek water. Samples (10 ml) of the diluted sediment were used in 50-ml serum bottles as described by Griffiths et al. (7), and duplicates were prepared at each concentration. The substrate was [U-¹⁴C]glucose at final concentrations of 25, 50, 100, and 200 μg/liter. The bottles were all incubated for the same time period (usually 5 h) at the environmental temperature. For most samples only the mineralized ¹⁴CO₂ was counted, but on one occasion the bacteria were filtered and counted as well.

**Chemical analyses.** The carbon and nitrogen concentrations in the sediments and raw substrates were determined in triplicate on dried, ground subsamples. The ash content of sediments was determined by heating oven-dried material of known weight in a muffle furnace at 550°C for 4 h. This method underestimates ash content slightly due to the water in clay lattices being driven off, but the error will be consistent and probably negligible. These chemical analyses were not performed on every sample. Aqueous suspensions (10%, wt/vol) of all four substrates were shaken for 24 h at 15°C and filtered through a glass fiber (GF/C [Whatman, Inc.]) disk, and the filtrates were analyzed for reducing sugars both spectrophotometrically and chromatographically. Ap-
proximate quantitative results were obtained by the dinitro-salicylic acid method and estimating the optical density at 540 nm (5). The qualitative analysis of free sugars was effected by a gas chromatographic method after the preparation of suitable derivatives (1). Tri-methylsilyl ether derivatives were made by dissolving the lyophilized extract in a mixture of pyridine and trimethylsilylimidazole (Tri-Sil Z; Pierce Chemical Co., Rockford, Ill.). The trimethylsilyl ether derivatives were injected onto one of a pair of matched OV17 columns operating differentially. The oven temperature was programmed to rise from 110 to 230°C at 4°C per min with 2 min at the initial temperature. The injector and detector temperatures were 190 and 260°C, respectively. Trimethylsilyl ether derivatives of pure sugars were used as standards.

RESULTS

Sediment gas production. Detectable rates of methanogenesis, nitrogen fixation, and CO₂ production were observed on each sampling occasion for amended and control sediments. Carbon dioxide production has been corrected for the quantity of CO₂ dissolving in the interstitial water by applying Henry's law to the equilibrium. The rates of all three processes stayed more or less constant with minor fluctuations in the unamended (control), P. menziesii bark, P. menziesii wood, and A. rubra bark pots (Fig. 1 and Table 1) throughout the 28-week sampling period. In contrast, the A. rubra wood pots showed a very marked peak in activity after 8 or 14 weeks, and gas production was always greater than in any of the other pots (Fig. 1). Analysis of covariance has been used to show that these three peaks are statistically significant. Thus, after testing for the equality of error variances the high values obtained from the A. rubra wood pots were compared with the values obtained from the amendment resulting in the next highest value at the same time. In each case the A. rubra wood results were significantly higher (P < 0.01).

The addition of either bark (both species) or P. menziesii wood had no effect on nitrogen fixation rates (Table 1), but did cause CO₂ production to rise approximately twofold above control levels in all three treatments. The amounts of ethylene produced in the absence of acetylene and contained within the acetylene were negligible. Methane production rates were more variable, so that the mean correlation coefficient from the combined regressions for methane production on each date was 0.776. For CO₂ production and nitrogen fixation the same mean correlation coefficients were 0.918 and 0.980, respectively. The rates of CO₂ production were 3 to 15 times higher than the rates of methane production (Table 1 and Fig. 1), but when these same results are expressed in terms of grams instead of moles of C the production rate of CO₂ was generally only about twice that of methane.

³¹⁴C uptake kinetics. The maximum transport rates (Vₘₐₓ) for glucose, calculated from ¹⁴C₂O₂ counts only, are shown in Table 2. Statistically significant regressions of tif on glucose concentration were obtained for every kinetic experiment, and the mean correlation coefficient was 0.986 (minimum, 0.959). With Spearman's rank test, Vₘₐₓ values from all of the treatments (except A. rubra wood) were shown to have a significant positive correlation with sediment temperature, which rose steadily from 6°C for the first to 14°C for the last sample (Table 2). Note that both A. rubra and P. menziesii wood pots had consistently lower Vₘₐₓ values than the control on the same date, and that both A. rubra and P. menziesii bark pots had consistently higher Vₘₐₓ values than pots amended with either wood (except for P. menziesii bark at 4 weeks).

Chemical analyses. The results of some chemical analyses are given in Table 3. The C/N ratios were reduced in all pots during the experimental period, but only the decrease in the control was statistically significant because of variability between replicates. The higher ash content of the control was expected due to the addition of organic material to all four of the remaining pots. The mean C/N ratios of the raw substrates before addition to the mud were 424, 60.6, 3,300, and 678 for A. rubra wood, A. rubra bark, P. menziesii wood, and P. menziesii bark, respectively. The silylated complex derived from the lyophilized alder wood extract included only one peak on the chromatogram not observed in any of the other extracts. This had a retention time

| Time in | A. rubra bark | P. menziesii bark |
| stream (wk) | CO₂ | C₂H₄ | CH₄ | CO₂ | C₂H₄ | CH₄ |
| 4 | 151 | 0.0807 | 27.2 | 132 | 0.0729 | 29.2 |
| 8 | 114 | 0.103 | 18.5 | 104 | 0.0931 | 25.1 |
| 14 | 118 | 0.107 | 11.3 | 107 | 0.0923 | 14.8 |
| 18 | 119 | 0.128 | 17.4 | 121 | 0.114 | 17.1 |
| 28 | 125 | 0.156 | 15.0 | 143 | 0.141 | 14.7 |

* Data are presented as nanomoles per gram (dry weight) per hour.
of 6.70 min and occurred on the tail of the solvent peak considerably ahead of any of the six sugars run previously as standards.

**DISCUSSION**

Good agreement has been shown between the rates of methanogenesis, nitrogen fixation, and CO₂ production for all four substrate additions and the controls. Only the *A. rubra* wood pots showed a pronounced peak in production rates after either 8 or 14 weeks of incubation, and these rates were always highest in the *A. rubra* wood pots, with only one exception (CO₂ production after 4 weeks). On the other hand, the results for *V̇ₘₐₓ* (which may be taken as a measure of general bacterial activity) did not conform to this pattern. Table 2 shows no peak for *V̇ₘₐₓ* in the *A. rubra* wood pots, and the results from these pots are consistently low compared with the others. The order of activity between treatments was not altered when incorporated as well as respired ¹⁴C was included in the calculation of *V̇ₘₐₓ* (after 28 weeks only).

The dissimilarity between the uptake kinetics and the other results may be due to the method used to determine *V̇ₘₐₓ*. Thus, the sediment was greatly diluted before the addition of the [¹⁴C]glucose, which may have disturbed it to such an extent that the results bore little resemblance to the activity occurring in the natural environment. Nevertheless, all sediments were diluted in the same way so that they should still be comparable with each other. It is also worth noting that the *V̇ₘₐₓ* values were determined under aerobic conditions, whereas methanogenesis and nitrogen fixation were determined anaerobically. In addition, although the tubes for CO₂ production were not gassed, anaerobic processes could have continued under the experimental conditions. Thus, the lack of agreement between the *V̇ₘₐₓ* results and the other activities may be a reflection of the incubation conditions. Nevertheless, the *V̇ₘₐₓ* results still conflict with the known faster decomposition of alder wood in aerobic streams compared with that of *P. menziesii* wood.

**Effect of bark.** There is extensive evidence to show that tannins inhibit microbial activity (4, 6), and *P. menziesii* bark is known to contain a high concentration of tannin (8%), sufficient for it to be used by a commercial leather manufacturer (14). Because the substrates were obtained from dead trees that had been lying in streams for some time it is possible that the tannins (which are water soluble) had been already leached out. Therefore, aqueous extracts of all of the substrates were tested for the presence of tannins by three separate reactions, i.e., with gelatin, ferric ammonium sulfate, and lead acetate solutions (15). The *P. menziesii* bark extract gave a strong positive reaction in each test, but none of the other substrates produced unequivocal results. Hence it is surprising that the *P. menziesii* bark pots gave such similar results in all respects to the *P. menziesii* wood and *A. rubra* bark pots. Tannins in *P. menziesii* bark are known to be attacked by certain fungi (16), but this is unlikely in the predominantly anaerobic conditions prevailing in our experiments. The moderate increases in methane and CO₂ production rates caused by bark are possibly due to the utilization of low-molecular-weight, water-soluble compounds, including several amino acids and sugars that are also known to be present in bark (10). These results suggest that *P. menziesii* bark has no adverse effect on microbial activity in stream sediments.

**Nitrogen fixation.** The results for nitrogen fixation have been given in units of ethylene (Fig. 1 and Table 1) because of the uncertainty associated with the conversion of acetylene reduced to N₂ fixed (8). In the *A. rubra* bark, *P. menziesii* wood, and *P. menziesii* bark pots, the rates of

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**TABLE 2.** *V̇ₘₐₓ* values for mineralized ¹⁴CO₂ from [¹⁴C]glucose after increasing periods in Berry Creek with the stated amendments

<table>
<thead>
<tr>
<th>Time in stream (wk)</th>
<th>Temp (°C)</th>
<th><em>V̇ₘₐₓ</em> values (ng [dry weight]/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>A. rubra</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wood</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>26.0</td>
</tr>
<tr>
<td>8</td>
<td>8.0</td>
<td>36.3</td>
</tr>
<tr>
<td>14</td>
<td>8.8</td>
<td>34.0</td>
</tr>
<tr>
<td>18</td>
<td>10.5</td>
<td>47.0</td>
</tr>
<tr>
<td>28</td>
<td>14.2</td>
<td>42.9</td>
</tr>
</tbody>
</table>

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**TABLE 3.** Chemical properties of stream sediments amended with woody materials

<table>
<thead>
<tr>
<th>Prepn</th>
<th>C/N</th>
<th>pH (28 wk)</th>
<th>% Ash content (18 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 wk</td>
<td>28 wk</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25.3</td>
<td>21.2</td>
<td>6.3</td>
</tr>
<tr>
<td><em>A. rubra</em> wood</td>
<td>32.9</td>
<td>29.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>A. rubra</em> bark</td>
<td>24.8</td>
<td>23.0</td>
<td>6.5</td>
</tr>
<tr>
<td><em>P. menziesii</em> wood</td>
<td>53.2</td>
<td>41.1</td>
<td>6.6</td>
</tr>
<tr>
<td><em>P. menziesii</em> bark</td>
<td>36.0</td>
<td>29.3</td>
<td>6.6</td>
</tr>
</tbody>
</table>
ethylen e production were never significantly different from the control, except for the P. menziesii wood sample after 4 weeks of incubation. This rate of approximately 0.1 nmol/g per h showed no large changes throughout the 28-week experimental period and contributed to the reduction in C/N ratios. It is similar to values obtained from the Tay Estuary, Scotland (9), but in comparable experiments, Tam et al. (18) could not detect any anaerobic N₂ fixation in stream sediment except when leaf material was added.

All of the added substrates were markedly deficient in nitrogen, so that any increase in the N₂ fixation rate might be expected to increase microbial activity generally. This was seen in the A. rubra wood pots, where the large increase in N₂ fixation was followed by peaks in methane and CO₂ production (Fig. 1). Although there is no direct evidence of a causal relationship, it is possible that increased N₂ fixation was the immediate cause of the much faster decomposition rate of A. rubra wood in streams compared with that of P. menziesii wood (2). What, then, makes A. rubra wood more attractive as a substrate for N₂ fixation compared with any of the other three substrates? It is most unlikely to be connected with the symbiotic nitrogen fixation which takes place in living A. rubra root nodules, and if the initial nitrogen content were the critical factor then P. menziesii bark might also be expected to promote fixation as it has about the same initial C/N ratio. One possible mechanism involves the energy source necessary for fixation. Nitrogen fixers cannot utilize cellulose, lignin, or other macromolecules, but the process of nitrogen fixation requires large amounts of energy, approximately 15 mol of ATP per mol of N₂ reduced. The most likely source of this energy is low-molecular-weight, readily metabolized molecules such as sugars, and certain sugars have been shown to promote N₂ fixation in sediments (12, 23).

If A. rubra wood could be shown to contain more sugar or a different type of sugar to the other three substrates then that may be the cause of the increased N₂ fixation. Both heartwood and bark from a number of trees are known to contain free sugars (10, 13, 21). All four filtrates contained appreciable quantities of reducing sugars, with the bark extracts having rather more than the wood extracts. Therefore, the quantity of sugar within the substrate did not seem to be the controlling factor, but it was still possible that the type of sugar might be important. Could the silyl derivative with a retention time of 6.70 min found only in the A. rubra wood extract be the substance which promoted N₂ fixation? It was evidently of smaller molecular size than common hexoses, and many possible compounds were silylated and chromatographed in the search for this unknown. The only substance which had an identical retention time was threitol, a four-carbon sugar alcohol.

Threitol was unexpected, as it had apparently not been previously recorded from plant material (3), and there remains the possibility that the unknown was not threitol because the retention time was the only positive information obtained. Nevertheless, five separate enrichments were then carried out in which threitol was added to mud from the same site at a final concentration of 0.59 mg/g (dry weight). Nitrogen fixation rates were monitored as before by the acetylene reduction assay. In only one of the five experiments did the threitol enhance fixation rates over the control, but in this single instance the fixation rate was 20 times that of the control. Hence, the role of threitol is not entirely clear, but in the apparent absence of any other energy source unique to alder wood we conclude that it may be involved in increased fixation rates shown to occur when A. rubra is added to stream sediment. However, the interaction is complex and not wholly mimicked by the simple addition of threitol.

In summary, some tentative conclusions concerning the microbial decomposition of woody materials in stream sediments may be derived from our experiments. From the combined CO₂ and CH₄ evolution rates A. rubra wood appears to decay at approximately four times the rate of A. rubra bark, P. menziesii wood, and P. menziesii bark. The hypothesis, frequently advanced (17), that the slower decomposition of certain woods is due to the presence of inhibitory substance(s), is not supported by our results. Faster decomposition of A. rubra wood may be related to enhanced N fixation, and tannins in P. menziesii bark (which were expected to inhibit microbial activity) were ineffectual. The energy source for the increased N₂ fixation has not been unequivocally identified, but there is some evidence in favor of threitol. These results do not allow accurate residence times of wood in stream sediments to be predicted because decay rates change with time. However, during the 6-month experimental period approximately 35% of the A. rubra wood is estimated to have been mineralized, compared with 10% of the other three materials. These estimates are consistent with previously published values obtained from direct measurements of dry weight loss (2).

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LITERATURE CITED


