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Bacterial Secondary Production on Vascular Plant Detritus: Relationships to Detritus Composition and Degradation Rate

MARY ANN MORAN* AND ROBERT E. HODSON

Department of Microbiology and Institute of Ecology, University of Georgia, Athens, Georgia 30602

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Bacterial production at the expense of vascular plant detritus was measured for three emergent plant species (*Juncus effusus*, *Panicum hemitomon*, and *Typha latifolia*) degrading in the littoral zone of a thermally impacted lake. Bacterial secondary production, measured as tritiated thymidine incorporation into DNA, ranged from 0.01 to 0.81 μg of bacterial C mg of detritus^{-1} day^{-1}. The three plant species differed with respect to the amount of bacterial productivity they supported per milligram of detritus, in accordance with the predicted biodegradability of the plant material based on initial nitrogen content, lignin content, and C/N ratio. Bacterial production also varied throughout the 22 weeks of in situ decomposition and was positively related to the nitrogen content and lignin content of the remaining detritus, as well as to the temperature of the lake water. Over time, production was negatively related to the C/N ratio and cellulose content of the degrading plant material. Bacterial production on degrading plant material was also calculated on the basis of plant surface area and ranged from 0.17 to 1.98 μg of bacterial C cm^{-2} day^{-1}. Surface area-based calculations did not correlate well with either initial plant composition or changing composition of the remaining detritus during decomposition. The rate of bacterial detritus degradation, calculated from measured production of surface-attached bacteria, was much lower than the actual rate of weight loss of plant material. This discrepancy may be attributable to the importance of nonbacterial organisms in the degradation and loss of plant material from litterbags or to the microbially mediated solubilization of particulate material prior to bacterial utilization, or both.

Emergent vascular plants account for a significant fraction of the primary production in shallow-water environments, such as wetlands and the littoral zones of lakes and ponds. Because carbon derived from these plants is difficult for most animals to assimilate, the bulk of vascular plant biomass is deposited as ungrazed material, to be processed largely via detrital food webs, originating with bacteria and fungi, rather than grazing food webs (31, 36). In wetland systems, bacteria are apparently the dominant degraders of vascular plant detritus (5, 6). Bacteria are able to utilize both the soluble fraction of the vascular plant (7, 16, 18), which rapidly leaches from the particulate material into the dissolved organic carbon pool following plant death, and the highly refractory lignocellulosic fraction (5, 6), which is deposited as particulate detritus. It follows, then, that bacterial decomposition of plant detritus may serve as a major pathway of carbon and energy flow in vascular plant-dominated systems. The fate of plant-derived carbon processed by bacteria includes mineralization via respiratory losses of bacteria, conversion into bacterial biomass, and, possibly, transfer to higher trophic levels in the aquatic food web via bacterivores (18, 35).

In view of the important role of bacteria in vascular plant degradation and the predominance of vascular plant detritus in shallow-water systems, it is of interest to quantify the rates of bacterial secondary production on detritus derived from leaves and stems of vascular plants. Despite the likely ecological significance of secondary production by bacteria attached to plant detritus, rates of this process have been measured in only a few instances, specifically for the bacterial community associated with standing dead *Spartina alterniflora* in coastal Georgia salt marshes (17; S. Y. Newell, R. D. Fallon, and J. D. Miller, Mar. Biol., in press), bacteria colonizing red mangrove leaves (1), stream bacterial communities (17a, 37), and bacteria on seagrass detritus (27). Furthermore, the relationships between bacterial production and the physical and chemical attributes of the plant detritus have not yet been explored, so that effects of differences in plant species, chemical composition of detritus, and stage of decomposition of detritus on the production of attached bacteria are not known. The latter factor is probably important in that, as decomposition progresses, the chemical composition of detritus derived from each species is expected to undergo significant changes; both the percent nitrogen and the percent lignin generally increase as plant-derived detrital material ages, while the C/N ratio decreases (35a, 38, 42).

If bacteria are the predominant degraders of vascular plant detritus in aquatic environments, as we currently suspect (5, 6), it follows that there should exist a direct and predictable relationship between the production of bacterial biomass and the rate of disappearance of the plant material which serves as the bacterial growth substrate. Quantifying bacterial production on vascular plant detritus would therefore allow a comparison of the calculated carbon demand by bacterial degraders with the measured carbon loss from the decomposing plant material.

In this study, we report rates of bacterial production, as determined by [*methyl*-^{3}H]thymidine ([^{3}H]TdR) incorporation into DNA, on plant detritus derived from three species of emergent vascular plants decomposing in the littoral zone of a thermally impacted reservoir in the southeastern United States. The three species, *Juncus effusus* L., *Panicum hemitomon* Schultes., and *Typha latifolia* L., differ substantially with regard to initial chemical composition, including lignocellulose, lignin, and nitrogen content. We investigate

* Corresponding author.

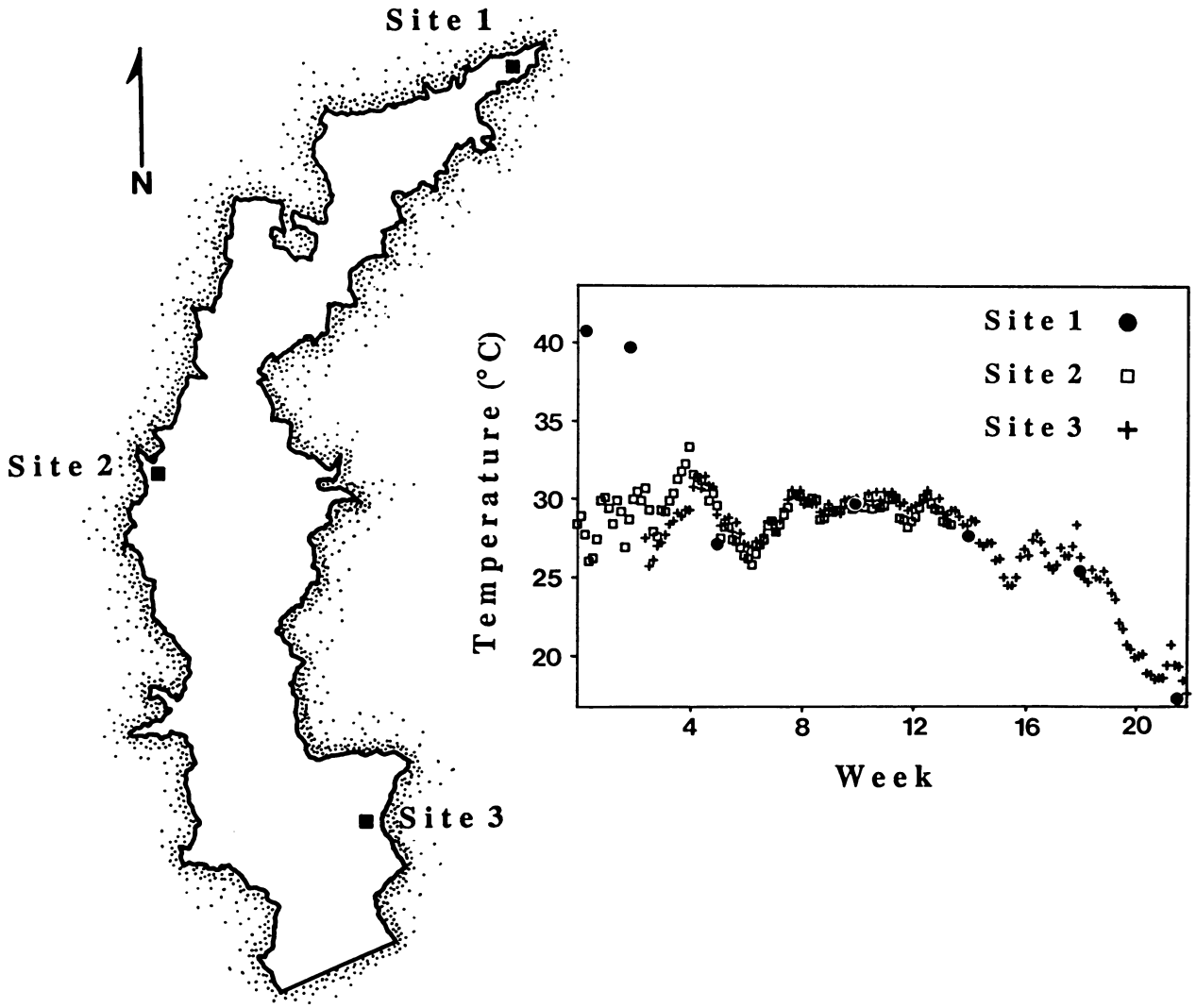


FIG. 1. Sampling locations at L Lake. The inset shows 10:00 am water temperatures at the three sites.

the relationship between bacterial production and initial differences in chemical composition, as well as subsequent responses of bacterial production to changes in the chemical composition of plant detritus through early diagenesis. We also examine the influence of temperature on the rate of bacterial production and, finally, determine whether secondary production of bacteria attached to plant detritus is directly related to the measured rates of weight loss for plant detritus decomposing in the lake.

MATERIALS AND METHODS

Site description. L Lake was established in 1985 to receive thermal discharge from the L Reactor nuclear materials production facility at the Savannah River Plant, S.C. Thermal effluent enters the lake at the north end and gradually cools as it passes southward through the 6-km lake basin. In early 1987, aquatic macrophytes were planted around the lake periphery in an effort to accelerate the natural development of littoral and wetland vegetation. Of the many successfully established species, three emergent macrophytes were chosen for measurement of bacterial production, the rush *J. effusus*, the grass *P. hemitomon*, and the cattail *T. latifolia*. These plants are all high in structural, or lignocellulosic, material, although the amount varies among the species; the lignocellulose content averages 88, 75, and 65% of the plant dry weight for *J. effusus*, *P. hemitomon*, and *T. latifolia*, respectively.

Three locations in littoral areas of the lake (water depth, approximately 1 m) that differed in thermal regime were chosen as sites for incubation of the plant material. Site 1 was located at the north end, 100 m from the reactor outfall, and experienced the highest temperatures during reactor operation (up to 42°C). Site 2 was located midway down the western shore of the lake, and site 3 was located at the south end of the lake on the eastern shore. Sites 2 and 3 had similar temperature regimes and were only minimally affected by the thermal discharge from the reactor (Fig. 1).

Litterbags. Above-ground portions of the plants were collected (while still green) in October 1987 from the south end of the lake, dried at 55°C, and cut into 6- to 8-cm pieces. Approximately 6 g of dried material was placed in litterbags (10 cm by 10 cm) made of fiber glass screening of mesh size 1 mm. Litterbags containing *J. effusus* were incubated at all three sites to investigate the effect of temperature on plant

decomposition, plant chemical composition, and bacterial secondary production. Litterbags containing *P. hemitomon* and *T. latifolia* were incubated only at site 3, to investigate intraspecies differences in these factors. In May 1988, litterbags were anchored to plastic pipes in the lake so that they remained suspended (10 to 30 cm) above the lake sediments. L Reactor was operating at this time, resulting in higher temperatures at site 1 than at the other two sites; the reactor was shut down for maintenance purposes 4 weeks later (25 June 1988) and remained out of operation for the remainder of the study. At intervals of 2 to 5 weeks, 12 litterbags containing decomposing *J. effusus* were removed (4 bags per site), and 4 bags each of *P. hemitomon* and *T. latifolia* were removed (site 3 only) for subsequent chemical characterization and determination of secondary production of attached bacteria.

Chemical characterization. Three of each set of four litterbags were used for analysis of the chemical composition of the plant detritus. The litterbags were rinsed thoroughly with tap water to remove trapped sediments; aquatic insects inside the bags (mainly the chironomid *Polypedilum* sp. and Odonate larvae) were removed by hand. Material was dried at 55°C and ground in a Wiley Mill to <425 μm in size. The ash content was determined by combusting subsamples at 550°C for 3 h. The content of lignocellulose, cellulose, hemicellulose, and lignin was determined by the detergent fiber method (23) by serially extracting plant material in a neutral detergent solution (nonlignocellulosic material is removed), an acidic detergent solution (hemicellulose is removed), and 72% sulfuric acid (cellulose is removed and lignin remains). Two replicate fiber analyses were performed on the contents of each litterbag, except for later collections (weeks 14, 18, and 22), when some bags contained only enough material for one analysis or when the contents of two or three bags were pooled to obtain sufficient material for a single analysis. The carbon and nitrogen content of the plant detritus was determined on a Perkin-Elmer 240C CHN analyzer. Results of all analyses were calculated on an ash-free basis.

Bacterial production. Bacterial production was determined by measuring the incorporation of [^{3}H]TdR into bacterial DNA, using a modification of the method of Fuhrman and Azam (20). Plant material from the remaining litterbag of each set of four was transported from the field in an insulated container filled with L Lake water at ambient temperature, and bacterial production was measured immediately upon return to the laboratory (within 5 h of collection). Plant culms were cut into 0.5- to 0.8-cm sections. Three sections were measured for length and diameter, minimizing disturbance to the plant pieces, and then placed in an acid-washed, autoclaved test tube containing 5 ml of filter-sterilized distilled water. For each plant species at each site, tubes containing three live replicate samples and one tube containing a killed control sample (0.5 ml of buffered Formalin added) were incubated at the temperature of the lake water at the time of collection (ambient-temperature treatment). An additional set of tubes was incubated at 25°C to control for temperature effects on bacterial production (constant-temperature treatment).

[^{3}H]TdR was added to each tube to give a final concentration of 10 nM. Following a 1.5-h incubation, 0.25 ml of 5 N NaOH was added to each tube, and after 15 min at room temperature, 1.4 ml of ice-cold 100% trichloroacetic acid was added and tubes were placed in an ice bath for 10 min (47). After extraction, the tubes were sonicated for 20 min in a sonicating bath containing ice-cold water to remove bacterial cells and associated macromolecules which may have remained stuck to the surface of the plant detritus. Following an additional 10 min in an ice bath, the contents of each tube were filtered through a cellulose acetate filter (pore size, 0.2 μm; Gelman GA-8 or MSI). The filters were rinsed four times with 2 ml of ice-cold 5% trichloroacetic acid, with the filter tower removed for the final rinse. The rinsed plant pieces were removed from the filter surface and placed in a scintillation vial with 10 ml of Scintiverse I (Fisher Scientific Co.) for scintillation counting. Radiolabel remaining associated with the plant detritus (termed unrecovered label) represented any unincorporated label or labeled macromolecules which remained attached to the surface of the plant detritus.

The filter (containing labeled protein, DNA, and possible lipid contaminants) was placed in a scintillation vial containing 2 ml of 5% trichloroacetic acid and hydrolyzed in a water bath at 100°C for 30 min. The vials were chilled for 15 min in an ice bath, and the contents were filtered through a second cellulose acetate filter. Following four rinses with ice-cold 5% trichloroacetic acid, the two filters were placed in a scintillation vial and dissolved in 1 ml of ethyl acetate. Scintillation cocktail (10 ml) was added to each vial, and radiolabel in this fraction (primarily protein) was quantified by liquid scintillation counting. The filtrate (containing primarily DNA) was collected, the total volume was determined, and a 1-ml subsample was placed in a vial with 10 ml of scintillation cocktail for counting. Unrecovered label (counts remaining on plant pieces) accounted for 12.7% ± 17.5% of the total incorporated or adsorbed counts following correction for Formalin-killed controls. Of the total recovered counts in macromolecules, the protein fraction accounted for 56.7% ± 16.8% and the DNA fraction accounted for 43.3% ± 16.8%.

Results from the three replicate tubes were averaged to give a single measure of [^{3}H]TdR incorporation for each plant species, at each site, at each time point. Because plant detritus samples for production measurements were taken from a single litterbag, we were interested in characterizing the extent of variability among litterbags in [^{3}H]TdR incorporation rates. In a separate experiment, three litterbags containing *J. effusus* detritus were incubated at each of two locations separated by 2 m at site 3. After 10 days of incubation, [^{3}H]TdR incorporation rates were determined for subsamples of plant detritus from each of the six bags as described above. No significant differences were found in the rates of [^{3}H]TdR incorporation into DNA between bags at the two locations (t test, $P > 0.05$). Furthermore, the variability among bags in [^{3}H]TdR in TdR incorporation rates was not any greater than the variability within the bags, since there was no significant difference in measured production among the six litterbags (analysis of variance, $P > 0.05$).

Because [^{3}H]TdR incorporation experiments were carried out with plant pieces suspended in distilled water (to eliminate the possibility that bacteria attached to plant detritus were growing at the expense of dissolved carbon in L Lake water), exogeneous sources of N and P were excluded. We were therefore concerned that nutrient limitation may have developed during the 1.5-h laboratory incubations. However, amendments with nitrate (ranging from 5 to 25 μM) and phosphate (ranging from 1 to 5 μM) did not result in any increases in bacterial incorporation rates compared with those in unamended water.

Bacterial production was calculated from the incorporation of [^{3}H]TdR into the DNA fraction only. Measurements

of the length and diameter of plant pieces were converted into surface area measurements by assuming that the pieces were cylindrical. Estimates of the weight of plant pieces were obtained by calculating a surface area-dry weight conversion factor with excess plant material from the litterbags at each time point. Pieces of each plant species (10 to 20 pieces) from each site were measured for length and diameter, dried at 55°C, and weighed, and the ratio of surface area to dry weight was calculated. As decomposition proceeded, the dry weight declined more rapidly for the plant detritus than did the surface area, as the plant culms maintained their shape while losing weight and turgidity. The rates of [^{3}H]TdR incorporation into bacterial DNA were calculated both on a surface area basis and on a dry-weight basis.

The reliability of the [^{3}H]TdR method for determining bacterial secondary production depends on whether bacterial uptake systems are saturated with respect to TdR and whether incorporation rates remain constant throughout the incubation period. Fallon and Newell (17) could not demonstrate saturation of TdR incorporation by bacteria attached to *S. alterniflora* detritus over a range of 10 nM to 100 μM TdR. However, our preliminary experiments demonstrated that rates of [^{3}H]TdR incorporation by bacteria attached to *J. effusus* detritus remained constant over a range of concentrations from 10 to 50 nM and therefore that bacterial uptake systems were probably saturated at the 10 nM [^{3}H]TdR concentration used for production measurements. Time course experiments demonstrated that incorporation rates remained linear for more than 2 h.

We chose to determine the relationship between [^{3}H]TdR incorporation into DNA and increases in bacterial biomass by using an empirically derived conversion factor (12, 28, 29), since fewer assumptions are involved with this approach. Water was collected from site 3 at the beginning and at the end of the litterbag incubation period (April and October 1988). A diluted natural bacterial culture was established by filter sterilizing 900 ml of water through 0.2-μm Nuclepore filters and adding it to a flask containing 100 ml of unfiltered water. The flask was incubated at room temperature with gentle stirring for 8 h (April) or 12 h (October). At the initial time point and at 2-h (April) or 3-h (October) intervals, water samples were withdrawn from the flask for determination of rates of [^{3}H]TdR incorporation. Simultaneously, duplicate 10-ml water samples were removed and preserved in 0.6 ml of Formalin for determination of changes in bacterial biovolume. Initial attempts were made to derive an empirical conversion factor based on the growth of bacteria attached to pieces of plant detritus. However, neither autoclaving nor sonicating plant pieces was successful in producing an actively growing attached bacterial population. Thus, we assume that the conversion factor calculated for bacteria growing in L Lake water is applicable to bacteria growing on plant detritus suspended in lake water.

Bacterial cells were counted by using acridine orange direct microscopy (24). Concentrated slides of stained bacteria were photographed with Ektachrome 400 film and projected onto a screen for measurement of cell lengths and widths (200 to 300 cells per time point). Cell sizes were calibrated by photographing and projecting fluorescent latex beads (Polysciences Inc.) with a known diameter of 1.70 μm. We also tried using beads closer to the size range of bacterial cells (0.25, 0.51, and 0.70 μm) but found the very bright fluorescent halo around the beads to be more problematic with smaller beads. Bacterial cell lengths and widths were converted to biovolume by using the formula for prolate spheres, $(4/3)\pi \cdot (L/2) \cdot (W/2)^2$, where L is the length of the cell and W is the width of the cell. We used the integrative method for calculating a conversion factor to relate the time course of [^{3}H]TdR incorporation to changes in bacterial biovolume (29, 39). The change in cell biovolume between the initial and final time points was divided by the total incorporation of [^{3}H]TdR into bacterial DNA over the same period (calculated by integrating [^{3}H]TdR incorporation rates). Conversions to bacterial carbon were made by assuming 2.2×10^{-13} g of bacterial C μm of cell volume^{-3} (9). The final [^{3}H]TdR conversion factors calculated were 0.550 (April) and 0.587 (October) μg of bacterial C pmol of [^{3}H]TdR incorporated^{-1}. A mean value of 0.569 μg of C pmol of [^{3}H]TdR^{-1} was used for all subsequent calculations.

Experiments were conducted to examine the effects of protozoans associated with the surface of plant detritus on measurements of bacterial production. Eucaryotic inhibitors (200 mg of cycloheximide liter^{-1} and 100 mg of colchicine liter^{-1} [40]) were added to the incubation water along with 10 μM ammonium and 1 μM phosphate. Following a 1-h incubation to allow the inhibitors to take effect, [^{3}H]TdR incorporation rates were determined and compared with those of unamended controls.

Statistical comparisons. The effect of location (site) on bacterial production was analyzed by one-way analysis of variance for each temperature treatment (ambient and constant) on production data from *J. effusus* detritus, the species incubated at all three sites. The effect of plant species on bacterial production was analyzed by one-way analysis of variance for each temperature treatment on production data from site 3, the site at which all plant types were incubated. The importance of water temperature, specific rate of weight loss of plant detritus, and chemical composition of plant detritus as predictors of bacterial production was determined by simple and multiple regression, and correlations among the variables were determined. Variables expressed on a percent or ratio basis (percent nitrogen, lignin, lignocellulose, cellulose, and hemicellulose and the C/N ratio) were all found to be normally distributed (Kolmogorov-Smirnov test), and therefore the regressions were performed on untransformed data. All analyses were run with production data expressed on a weight basis (per milligram of plant detritus) and on a surface area basis (per square centimeter of plant detritus).

RESULTS

Plant decomposition. Vascular plant detritus was rapidly lost from litterbags in L Lake (Fig. 2). By week 22, no detectable detritus remained in the litterbags originally containing *J. effusus* at sites 2 and 3 and *T. latifolia* at site 3. However, weight loss was slightly slower for both *J. effusus* at site 1 and *P. hemotomon* at site 3, with 17 and 16%, respectively, remaining after 22 weeks of decomposition. Effects of location within the lake on weight loss from the litterbags, potentially reflecting the different temperature regimes at the three sites, were not easily discerned. *J. effusus* detritus lost weight most slowly at the highest-temperature location (site 1) and most rapidly at the midlake location (site 2). Reactor-induced differences in thermal conditions were present only during the first 4 weeks of decomposition, during which time the sites differed by as much as 13°C; thereafter, temperatures at the three sites were within 1°C at the time of each collection (Fig. 1). *Polypedilum* sp., a bright-red chironomid which burrows through plant stems, was found in and on the plant detritus

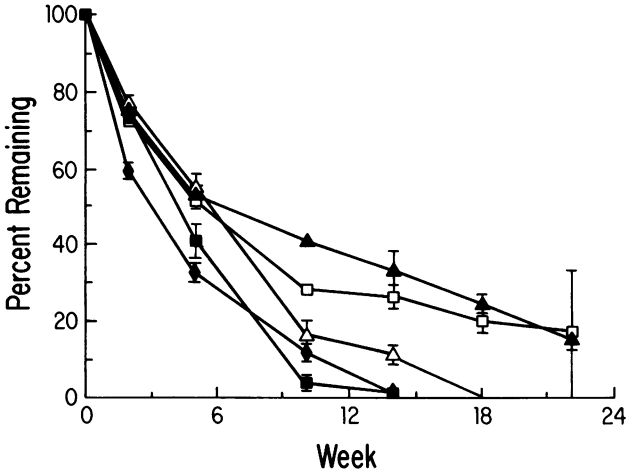


FIG. 2. Weight loss of plant material from litterbags in L Lake (n = 3; bars give the standard error). Symbols: □, *J. effusus*, site 1; ■, *J. effusus*, site 2; △, *J. effusus*, site 3; ▲, *P. hemitomon*, site 3; ◆, *T. latifolia*, site 3.

and was particularly abundant at sites 2 and 3. This insect larva creates tunnels for filter feeding in the plant stems and possibly feeds on the plant tissue as well (8, 45). The shredding behavior of *Polypedilum* sp. makes it possible that total weight loss from the litterbags reflected insect-mediated loss of small particles of plant detritus through the mesh bags as well as microbial decomposition losses.

Degradation-dependent changes in plant chemistry. The composition of the plant detritus changed predictably as degradation in L Lake proceeded. As has been found in many previous studies of vascular plant decomposition (see, e.g., references 35a, 38, and 42), the percent nitrogen in the detritus increased with time (Fig. 3), possibly representing accrual of nitrogen-rich microbial biomass, humification processes, or nitrogen fixation. Correspondingly, the C/N ratio of remaining plant material decreased as decomposition proceeded (Fig. 3). Changes in nitrogen content were most pronounced for *J. effusus* at site 2 and for *T. latifolia* and were least evident for *P. hemitomon*, initially the most nitrogen poor of the three plant species.

The total lignocellulose content of the three species did not show a substantial change with time, except for an initial increase in the percentage of lignocellulose in *P. hemitomon* and *T. latifolia*, reflecting the leaching losses of nonlignocellulosic components of the plant material during the first 2 weeks of incubation (Fig. 4). The lignin content, however, gradually increased with time for all species at all sites (Fig. 4). This phenomenon of relative enrichment in lignin of degrading vascular plant detritus has been found previously (22, 25, 35a, 42, 48) and probably reflects the discrimination against the lignin component of plant detritus during microbial decomposition. The two polysaccharide components of lignocellulose, cellulose and hemicellulose, exhibited contrasting dynamics with time. The relative cellulose content of the plant material generally decreased as decomposition progressed, with the changes being most pronounced for *J. effusus* at site 2 and least pronounced for *P. hemitomon* (Fig. 4). This preferential utilization (or removal) of the cellulose fraction of lignocellulose relative to the other structural components (hemicellulose and lignin) has been found previously for aquatic vascular plant material degrading in wetland ecosystems (35a). The hemicellulose content

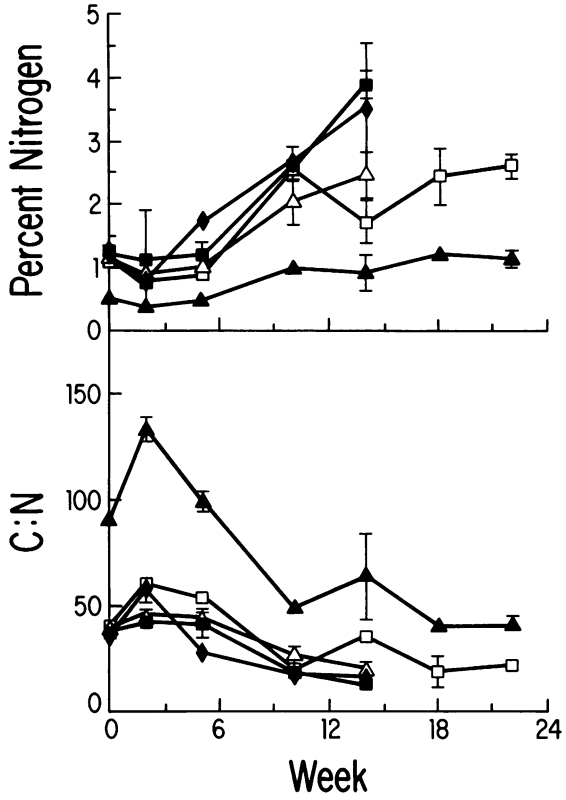


FIG. 3. Percent nitrogen (top) and C/N ratio (bottom) of degrading plant material in L Lake. Symbols are as in Fig. 2 (n = 3 except for some samples at week 18, for which n = 2).

showed no clear trends with time (Fig. 4) and appeared to constitute a nearly constant proportion of the plant detritus.

Site effects on bacterial production. Bacterial production on vascular plant detritus, considering all sites and all time points, varied from 0.01 to 0.81 μg of bacterial C mg of detritus^{-1} day^{-1} (weight-based calculation; Fig. 5) and from 0.17 to 1.98 μg of bacterial C cm^{-2} day^{-1} (surface area-based calculation). The location in L Lake at which the plant detritus was incubated had a significant effect on measured bacterial production, since [^{3}H]TdR incorporation rates supported by the bacterial assemblages attached to *J. effusus* detritus were significantly different among the three sites for the ambient-temperature treatment ($P < 0.05$). For the constant-temperature treatments, which eliminated temperature variability among sites for the duration of the [^{3}H]TdR incorporation experiments, bacterial production was not significantly different among sites. Average production (both temperature treatments combined) at sites 1, 2, and 3 was 0.12, 0.34, and 0.13 μg of C mg of detritus^{-1} day^{-1}.

Plant species effects on bacterial production. The production of attached bacteria was also significantly affected by the plant species from which the detritus was derived (Fig. 5). The average bacterial production on *J. effusus*, *P. hemitomon*, and *T. latifolia* detritus at site 3 (two temperature treatments combined) was 0.13, 0.04, and 0.25 μg of bacterial C mg of detritus^{-1} day^{-1}, respectively, and differences among the plant species were significant both for ambient-temperature incubations ($P < 0.01$) and constant-temperature incubations ($P < 0.01$). Differences in the ability of various types of plant detritus to support bacterial production may be related to the initial chemical composition of the

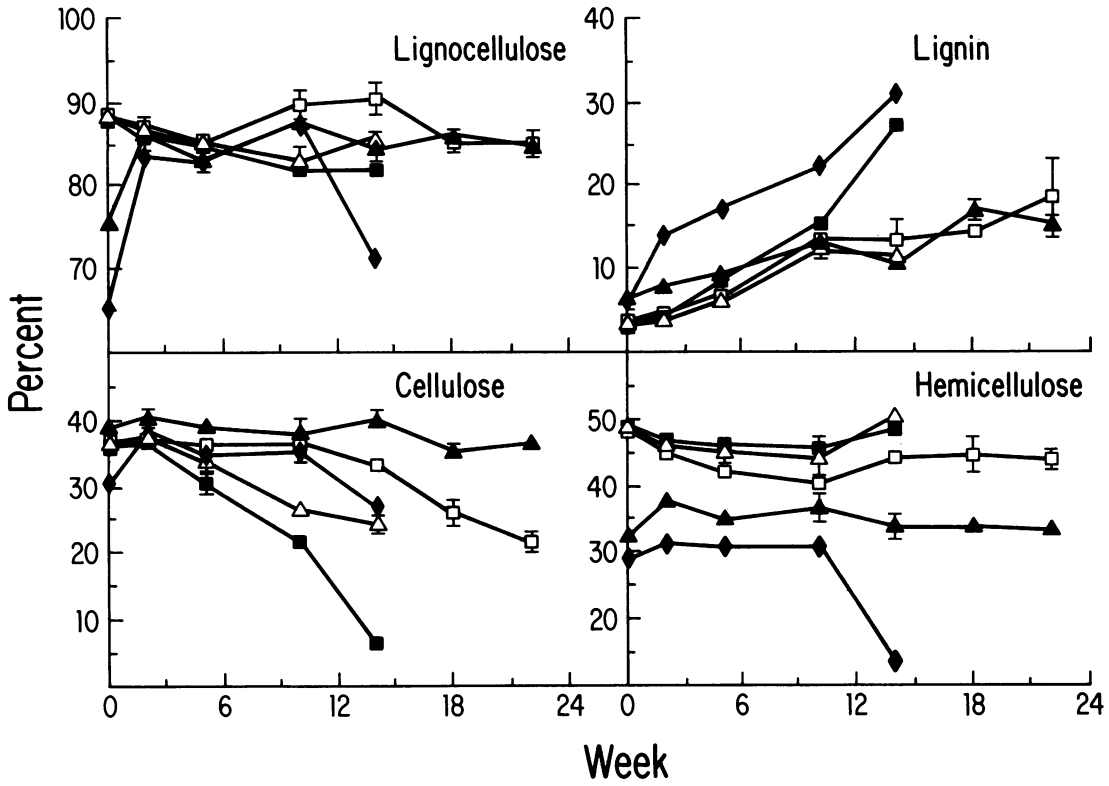


FIG. 4. Chemical composition of degrading plant material in L Lake. Symbols are as in Fig. 2 (n = 6 except for some samples at weeks 14, 18, and 22, for which n = 1 to 5).

plants (Fig. 3 and 4), since the three species differ in nitrogen content (1.1% for fresh *J. effusus* material, compared with 0.5% for *P. hemitomon* and 1.3% for *T. latifolia*), C/N ratio (40.6, 90.4, and 36.4, respectively), lignin content (3.4, 6.4, and 5.8%, respectively), lignin/N ratio (3.1, 12.8, and 4.5, respectively), and lignocellulose content (88.2, 75.3, and 65.5%, respectively).

Interspecies differences, however, were apparent only when bacterial production was calculated on a weight basis. On a surface area basis, production of detritus-attached bacteria did not differ among the three species (0.56, 0.43, and 0.42 μg of C cm^{-2} day^{-1}). Our measurement of surface area is possibly not an appropriate representation of the area available to bacterial cells, since the microscopic topography of the detritus may be unrelated to the macroscopic linear measurements made on pieces of plant stems. Nonetheless, expressing the production of attached bacteria on the basis of macroscopic surface area of the detritus apparently eliminates differences among plant species. The ratios of (nominal) surface area (in square millimeters) to weight (in milligrams) for fresh plant material of the three species were 18 (*J. effusus*), 6 (*P. hemitomon*), and 49 (*T. latifolia*).

Predictors of bacterial production on plant detritus. Environmental and chemical composition variables were tested as predictors of bacterial production on vascular plant detritus. Several of these were not independent measures (Table 1). Although water temperature was unrelated to any other factor, chemical composition variables were correlated among themselves. The nitrogen content and the lignin content were positively correlated with one another, but negatively correlated with the C/N ratio, lignocellulose content, and cellulose content of the plant detritus. The specific rate of weight loss (milligrams lost per milligram remaining per day) (analogous to calculating a decomposition constant [k] for each sampling interval) increased during decomposition, an unusual pattern (35a) which may result from insect-mediated, rather than microbially mediated, losses of plant material. The specific rate of weight loss was positively correlated with the percentage of lignin and negatively correlated with the percentage of lignocellulose.

Simple regressions showed bacterial production to be significantly related to temperature, specific rate of weight loss, nitrogen content, C/N ratio, lignin content, and cellulose content for weight-based production calculations and to temperature, cellulose content, and hemicellulose content for surface area-based production calculations (Table 2). As all species at all sites were grouped, these analyses encompass the effects both of inherent differences among plant species with regard to initial chemical composition and differences with time within each species related to decomposition-mediated changes in chemical composition. In general, production by attached bacteria increased as plant detritus aged, for production expressed both on a surface area basis and, more so, on a weight basis (Fig. 5, 25°C data; slopes significantly different from zero for *J. effusus* at sites 2 and 3). Thus, production was negatively related to variables which decreased as the detritus aged (percent cellulose and C/N ratio) and positively related to variables which increased as the detritus aged (percent lignin and percent nitrogen). Multiple regression techniques were used to identify the factors most useful in predicting bacterial production on plant detritus. For bacterial production calculated on a weight basis, the cellulose content of the plant detritus, water temperature, and specific rates of weight loss were all important variables in predicting bacterial production [production = −0.018 − 0.012(percent cellulose) + 0.019

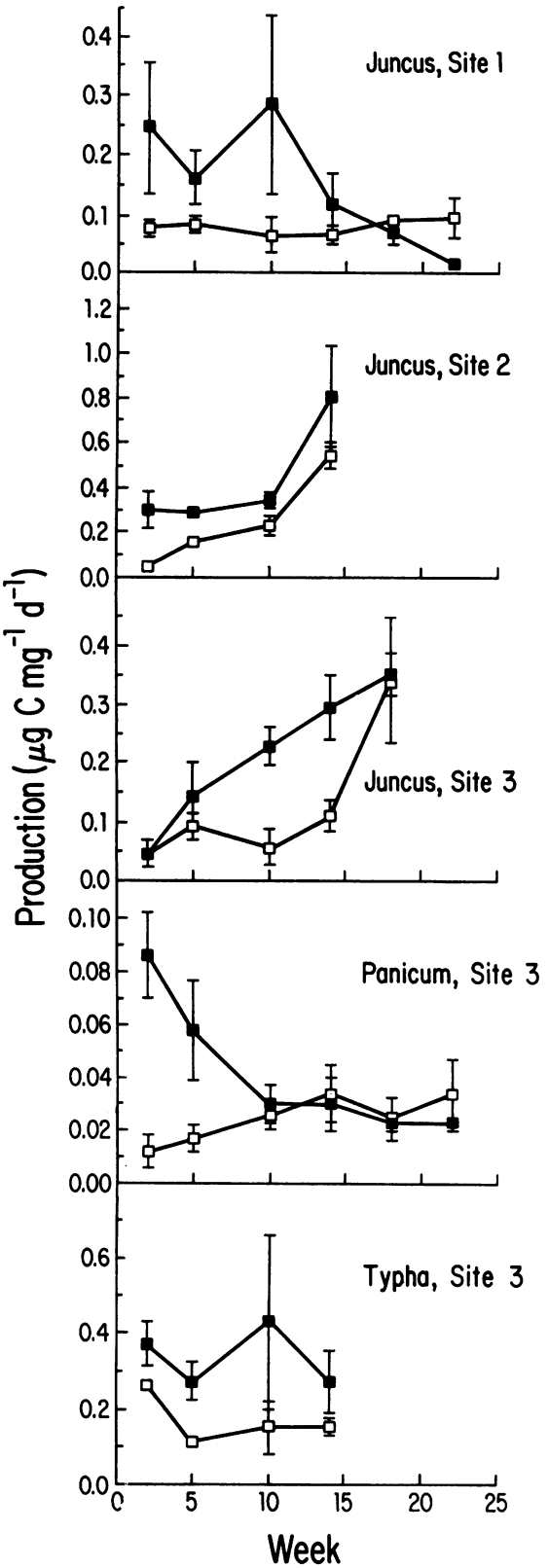


FIG. 5. Rates of bacterial production on vascular plant detritus (weight basis) at ambient lake temperatures (■) and at 25°C (□) (n = 3; bars give the standard error).

(temperature) −1.99(weight loss)], explaining 63% of the variance associated with production measurements. For bacterial production calculated on a surface area basis, cellulose content, temperature, and hemicellulose content were significant predictors of production [production = −1.16 + 0.074(temperature) − 0.024(percent cellulose) + 0.014(percent hemicellulose)], explaining 56% of the measurement variance. Variables relating to nitrogen dynamics in plant detritus (percent N and C/N ratio), although individually correlated with bacterial production, did not contribute significantly to the multiple regression equation, owing to the high correlation of N-related variables with the cellulose content of the plant detritus.

DISCUSSION

Previous studies on the decomposition of vascular plant material have suggested criteria by which to predict the biodegradability of vascular plant detritus. Decomposition losses are likely to be greater for plant species which are high in initial nitrogen content (4, 32), low in initial lignin content (19), and low in structural (or lignocellulosic) material (22); have a low C/N ratio (43); and have a low lignin/N ratio (4, 34). According to these criteria, of the three plant species chosen for this study, *P. hemitomon* would be expected to degrade the most slowly, owing to its low initial nitrogen content and high lignin/N ratio. Ordering *J. effusus* and *T. latifolia* is not straightforward, since *J. effusus* has the lowest lignin content and lignin/N ratio of the three species while *T. latifolia* has the highest nitrogen content, lowest C/N ratio, and lowest lignocellulose content. Weight loss data from site 3 indicate that *P. hemitomon* is indeed the least biodegradable of the three species, followed by *J. effusus* and then *T. latifolia*.

Factors which are good predictors of the biodegradability of plant detritus might also be expected to be good predictors of growth by attached bacterial cells. Rates of [^{3}H]TdR incorporation measured in this study indicate that initial nitrogen content, lignin content, and C/N ratio are good predictors of subsequent bacterial production, since *P. hemitomon* supported the lowest bacterial production (on a weight basis) throughout decomposition and *T. latifolia* supported the highest. However, these interspecies differences disappear when production is expressed on a surface area basis, possibly indicating that the available surface of particulate detritus, rather than detritus quality, is the primary factor controlling bacterial activity and subsequent detritus decomposition. This explanation, however, assumes that the method used to measure the two-dimensional surface area gives a reasonable approximation of the actual three-dimensional surface area available to bacteria. In addition, it assumes that pitting and disruption of the plant stem surface as a result of the decomposition process, which will increase the surface area available to bacterial cells, is constant across all three species.

The temperature of the water in which the plant material was incubating was one of the important predictors of bacterial growth, whether production was expressed on a weight basis or on a surface area basis. This is an expected result and is in accordance with the known importance of temperature in determining rates of plant decomposition in aquatic ecosystems (3, 11, 46). That intersite differences in bacterial production (site 2 > site 3, site 1) did not correlate directly with the temperature regime at the sites (site 1 > site 2, site 3) may be attributable to the fact that temperature differences between sites were pronounced only during the

TABLE 1. Correlation coefficients among environmental and plant composition variables

Variable	Correlation coefficient[a] with:							
	Temp	Wt loss	% N	C/N	% Lignocellulose	% Lignin	% Cellulose	% Hemicellulose
Temp	1.00							
Wt loss per day	0.27	1.00						
% N	−0.17	0.51	1.00					
C/N	0.24	−0.28	−0.79**	1.00				
% Lignocellulose	0.04	−0.78**	−0.40	0.16	1.00			
% Lignin	−0.28	0.52*	0.80**	−0.53*	−0.56*	1.00		
% Cellulose	0.19	−0.43	−0.82**	0.63*	0.36	−0.56*	1.00	
% Hemicellulose	0.09	−0.37	−0.05	−0.09	0.55*	−0.54*	−0.33	1.00

[a] *, Significant correlation at $P < 0.05$; **, significant at $P < 0.01$.

first 4 weeks of the study, that the extreme temperatures of site 1 (>40°C) were actually inhibitory to bacterial activity, or that other variables were interacting with temperature to influence bacterial growth in a more complex manner.

Although initial chemical composition is apparently a good predictor both of relative biodegradability and relative weight-based production by attached bacteria for these three plant species in L Lake, the chemical composition of each plant changed from its initial composition as the study progressed and the detritus aged. When current chemical composition was related to bacterial production, we found the nitrogen and lignin content to be positively related to bacterial production throughout the decomposition of the plant detritus and the cellulose content and C/N ratio to be negatively related to production. The positive correlation of production with lignin content is unexpected, since lignin is a highly refractory biopolymer (14, 49) and rates of vascular plant decomposition are generally inversely correlated with the percentage of lignin (19, 33). Moreover, the initial lignin content appears to be negatively related to the average rate of bacterial production supported by the three plant species. The positive relationship between nitrogen content and production is not necessarily indicative of increased nitrogen availability to bacterial degraders, since highly refractory products of humification may account for a large fraction of the detrital nitrogen (38, 42).

Secondary production of attached bacteria might be expected to be directly related to rates of detritus decomposition and therefore to rates of weight loss of the detritus. Rates of bacterial production on the three plant species correlated well with their weight loss rates, in that ordering of the plants was *P. hemitomon* < *J. effusus* < *T. latifolia* for both measures. In addition, results from the regression analyses indicate that calculated bacterial production rates and measured weight loss are positively correlated. The specific rate of weight loss was a significant predictor of bacterial production in a simple regression (weight basis only), although the r^2 value indicates that it explains less than 30% of the variation in production; weight loss was also a significant variable in the multiple regression (weight basis only).

TABLE 2. Results of simple regressions of bacterial production with environmental and plant biochemical variables

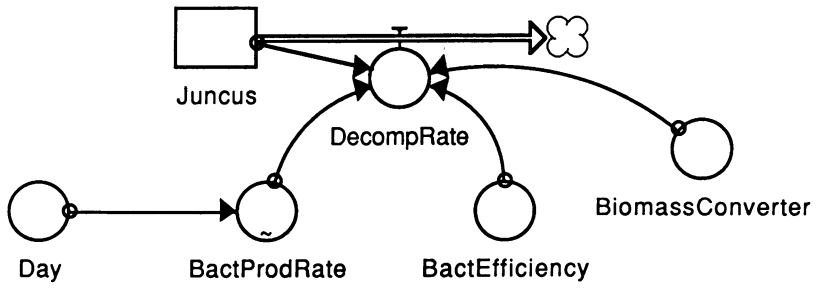
Variable	Wt basis		Surface area basis	
	r^2	Slope[a]	r^2	Slope[a]
Temp	0.14	0.018**	0.27	0.070**
Wt loss per day	0.28	4.86**	0.06	7.07
% N	0.31	0.098**	0.03	0.113
C/N	0.13	−0.003**	0	−0.001
% Lignocellulose	0.06	−0.013	0	−0.004
% Lignin	0.18	0.011**	0	0
% Cellulose	0.38	0.013**	0.16	−0.026**
% Hemicellulose	0	0.002	0.15	0.024**

[a] **, Slope significant at $P < 0.01$.

We further investigated the potential relationship between bacterial production and detrital weight loss by comparing measured specific rates of weight loss from the litterbags (milligrams of material lost per milligram of material remaining per day) with bacterial carbon demand calculated from [^{3}H]TdR incorporation rates. Provided that we have a reasonable estimate of the carbon conversion efficiency of bacteria growing at the expense of vascular plant carbon, production measurements can be used to estimate the carbon demand of bacterial decomposers and therefore the contribution of bacteria to detritus weight loss. A simple simulation model (Stella, High-Performance Systems; Fig. 6) was used to generate a weight loss curve from the bacterial production measurements on *J. effusus* at site 1. Bacterial production (micrograms of bacterial C per milligram of detritus per day) was assumed to be 30% of the total bacterial carbon utilization (2). Carbon utilization rates were converted to bacterial organic matter utilization rates by assuming a 47% carbon content of *J. effusus* tissue. Rates of bacterially mediated decomposition calculated for each sample date (2, 5, 10, 14, 18, and 22 weeks) were interpolated between points to estimate daily rates of *J. effusus* detritus utilization by attached bacteria, and, by using these daily rates, a curve representing bacterial contributions to weight loss was generated. Comparison of the model-generated curve with actual weight loss from litterbags (Fig. 6) shows that bacterial production measured by following our [^{3}H]TdR protocol is sufficient to account for only a small fraction of actual loss of *J. effusus* detritus, since the model predicts that 83% of the plant material remains after 150 days of decomposition, compared with the measured value of 17%. The disparity between curves can be interpreted in three ways: as evidence for an insignificant role for bacteria in the decomposition of vascular plant detritus in this lake (and therefore an important role for nonbacterial organisms or physical processes), as evidence for microbially mediated solubilization (leaching or lysis) of a significant fraction of particulate plant detritus prior to bacterial utilization, or as evidence for problems in using the [^{3}H]TdR method for measuring bacterial production at the expense of particulate detrital plant material.

Although evidence has often suggested that bacteria play a secondary role to fungi in the decomposition of vascular plant detritus in terrestrial ecosystems (13, 30), recent data

A



B

Juncus = Juncus + dt * (-DecompRate)
INIT(Juncus) = 100
BactEfficiency = 0.30
BiomassConverter = 2.128
Day = TIME
DecompRate = ((BactProdRate*Juncus)/BactEfficiency)*BiomassConverter
BactProdRate = graph(Day)
(0.0,0.000245),(15.00,0.000245),(30.00,0.000162),(45.00,0.000162),
(60.00,0.000285),(75.00,0.000285),(90.00,0.000118),(105.00,0.000118),
(120.00,0.0000710),(135.00,0.0000710),(150.00,0.0000170)

C

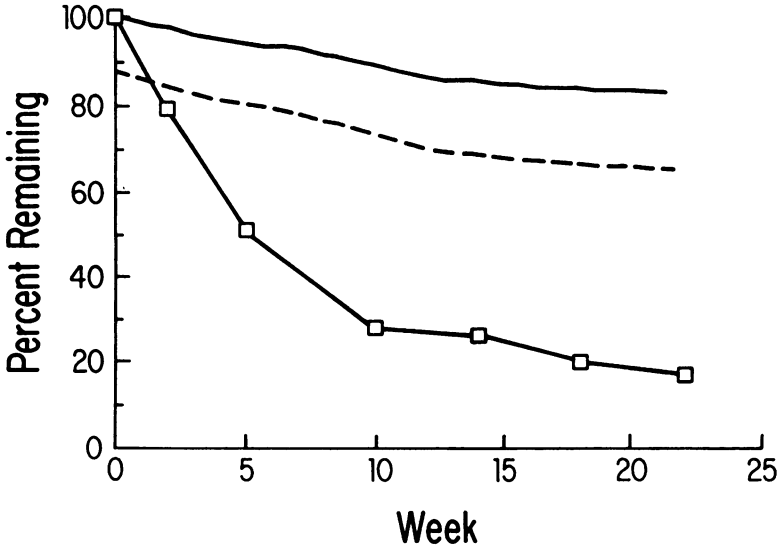


FIG. 6. Stella (High-Performance Systems) simulation model based on *J. effusus* decomposition at site 1. (A) Model diagram. (B) Model equations. (C) Model-generated estimate of bacterial detritus utilization (——) compared with measured weight loss of detritus (□——□). Model prediction of bacterial decomposition corrected for solubilization of detrital material are also shown (– – –).

indicate that bacteria may be the major mediators of vascular plant decomposition under submerged conditions (5, 6). Using both eucaryotic inhibitors and size fractionation methods to exclude fungi, Benner et al. (5) found rates of lignocellulose decomposition by bacteria to average 85% of rates mediated by the full microbial community. Inactivation of bacteria by a procaryotic inhibitor, however, resulted in decomposition losses amounting to only 28% of the control (5). Findlay and Arsuffi (17a) calculate that there is roughly equal carbon demand by bacterial and fungal colonizers of stream leaf detritus. Although experiments to discriminate bacterial from nonbacterial activity in the decomposition of lignocellulosic detritus in L Lake have not been carried out, we assume that pathways of plant detritus transformation in this lake are not remarkably different from those in previously studied aquatic environments. Although the relative roles of bacteria and fungi in lignocellulose decomposition in aquatic environments remain controversial, the most direct evidence thus far indicates a very important, if not dominant, role for bacteria.

An alternative cause of the discrepancy between measured weight loss and calculated bacterial production-based decomposition rates is the activity of nonmicrobial organisms, principally insects, associated with the plant detritus. Larvae of the chironomid genus *Polypedilum* were extremely abundant in the litterbags, carving out feeding tunnels through the upper cell layers of the plant stems and possibly consuming plant tissue as well (8, 45). The activity of this animal conceivably resulted in either direct ingestion of plant material or extensive shredding of plant tissue and subsequent loss of small pieces through the 1-mm litterbag mesh. The kinetics of weight loss from the litterbags is not inconsistent with the hypothesis that a nonbacterial component is important in the disappearance of the plant material. Whereas a decreasing or constant specific rate of decomposition is the kinetic pattern normally seen for microbial decomposition of vascular plant material (5, 35a), an increasing specific rate was found for vascular plant material degrading in L Lake, with the increase coinciding temporally with the appearance of the abundant insect fauna.

In determining the production of bacteria growing at the expense of plant detritus, errors can be introduced when converting rates of [^{3}H]TdR incorporation into carbon equivalents. Methods for calculating bacterial production from [^{3}H]TdR incorporation rates are numerous, and no consensus currently exists with regard to a single, most appropriate method. Coveney and Wetzel (12) calculated 15.2×10^5 μm^3 of bacterial biovolume pmol of [^{3}H]TdR incorporated into DNA^{-1} for a freshwater lake; this is within a factor of 2 of the empirical value calculated in this study (equivalent to 25.9 μm^3 $pmol^{-1}$). Our L Lake conversion factor can be converted to a cell number basis by assuming a representative cell volume of 0.26 μm^3 (the average volume throughout the conversion factor experiments ranged from 0.10 to 0.46 μm^3). This value of 9.9×10^{18} cells mol of $[^3H]TdR^{-1}$ is fivefold higher than the frequently cited value of 2.0×10^{18} cells mol^{-1} (20) but is well within the range reported in the literature (15, 41) and is similar to, but twofold higher than, the only factor calculated for bacterial growth on plant detritus, 4.0×10^{18} cells mol^{-1} for growth on *S. alterniflora* detritus (17). Thus, our empirically determined factor is at the high end of the range of values found for many other systems, and its use is unlikely to result in significant underestimation of production of bacterial carbon. The simulation model predicts that the L Lake conversion factor would have to be increased approximately 10-fold for calculated bacterial carbon demand based on [^{3}H]TdR incorporation to match measured losses of plant detritus.

Provided that the [^{3}H]TdR conversion factor used in this study is reasonable, underestimates of bacterial production may nonetheless result if rates of DNA synthesis under laboratory conditions do not reflect natural rates of synthesis in L Lake. We have considered two possible mechanisms by which rates of [^{3}H]TdR incorporation in the laboratory may have misrepresented in situ bacterial production and resulted in the discrepancy between measured rates of weight loss and predicted weight loss based on observed bacterial production. (i) If bacterivory were significant during the 1.5-h incorporation experiments, ^{3}H-labeled bacterial biomass consumed by protozoans might be incorporated into non-DNA protozoan biomass, lost as protozoan respiratory products, or released as non-DNA dissolved organic compounds, all of which would result in underestimation of bacterial growth. However, we discount this possibility, since additions of eucaryotic inhibitors with and without nutrient amendments to control for regeneration activity of the protozoans did not change the rates of [^{3}H]TdR incorporation (data not shown). (ii) If the veneer of active bacteria associated with degrading particulate material is fragile, with cells being loosely associated with the detritus rather than firmly attached, removal of plant detritus from the lake followed by transport and experimental manipulations (sectioning of plant material and surface area measurements) might result in the loss of a significant fraction of attached bacteria prior to measurements of [^{3}H]TdR incorporation. We were unable to test this possibility by characterizing the mode of attachment of bacterial cells in situ, but we suggest the idea of a fragile veneer as a possible mechanism for the underestimation of bacterial production on plant detritus. Bacterial production measured in this study (0.01 to 0.81 μg of C mg^{-1} day^{-1}) is somewhat lower than production measured on fresh leaf detritus (up to 4.5 μg of C mg^{-1} day^{-1} [17a]) and on fresh seagrass detritus (1.1 μg of C mg^{-1} day^{-1} [27]) but comparable to bacterial production on older, standing dead grass leaves (0.18 μg of C mg^{-1} day^{-1}; R. D. Fallon and S. Y. Newell, personal communication).

A further possible cause of the discrepancy between bacterial production measurements and detritus weight loss, and perhaps one of the most likely, is the conversion of particulate detrital material into dissolved detrital material prior to its utilization by bacteria. Nonlignocellulosic components of plant material (simple sugars, amino acids, proteins, lipids, etc.) rapidly leach from plant material following its deposition as detritus (7, 21, 44). As leaching of submerged plant detritus is generally complete within the first 2 weeks of decomposition (35a), nonlignocellulosic compounds were lost from the litterbags prior to the first sampling and therefore their contribution to bacterial growth was not measured. Moreover, recent work with particulate material in the ocean (10, 26) and particulate lignocellulose in several wetland ecosystems (M. A. Moran and R. E. Hodson, Limnol. Oceanogr., in press) indicates that a sizeable percentage of insoluble particulate organic carbon is converted to dissolved organic carbon (via microbial activity) prior to utilization by freeliving and/or attached bacteria. Moran and Hodson (in press) estimate that a minimum of 10 to 40% of particulate lignocellulose is utilized by bacteria via this pool of solubilized compounds. Since utilization of dissolved organic carbon thus produced could not be tracked by our experimental design, a significant proportion of bacterial growth at the expense of vascular plant detritus was probably overlooked. Recognizing that bacterial pro-

duction on the nonlignocellulosic components of plant material was not measured, and assuming that production on the lignocellulosic components was underestimated by 40%, a second decomposition curve was generated by the simulation model (Fig. 6). In this case, 66% of *J. effusus* detritus is predicted to remain after 22 weeks of decomposition in L Lake, compared with the measured value of 17%. Provided that our estimates of bacterial production and correction for solubilization of particulate detritus are reasonable, this revised model predicts that approximately 40% of the weight loss of *J. effusus* is attributable to bacterial decomposition of the plant detritus, whereas disappearance from the litterbags of the remaining 60% is due to nonbacterial factors.

We conclude that bacterial production (measured as [^{3}H]TdR incorporation into DNA) by bacteria attached to plant detritus in L Lake correlates well with factors which have long been assumed to influence decomposition rates of vascular plant material in aquatic environments; these include nitrogen content, C/N ratio of the plant detritus, and temperature. An additional factor examined in this study, cellulose content, is apparently also a good predictor of bacterial production on vascular plant-derived carbon. However, despite the statistical relationship with environmental and plant biochemical parameters, the calculated bacterial carbon demand based on [^{3}H]TdR incorporation rate is sufficient to account for only a portion of the measured weight loss of plant material degrading in L Lake. The most likely reasons for this discrepancy are the shredding activity of the abundant insect community, which potentially caused nonbacterially-mediated losses of plant detritus, and the conversion of particulate detritus into dissolved forms prior to bacterial utilization.

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