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Microbial Colonization and Decomposition of *Carex* Litter in an Arctic Lake

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The decomposition and microbial colonization of *Carex* leaf litter were examined in an arctic lake in Alaska during the summer of 1978. Dried leaf segments in screen bags were placed at various locations and depths for 13 and 26 days. Weight loss varied from 24.15 to 33.56% and from 27.69 to 65.01% after 13 and 26 days, respectively. Abiotic controls lost approximately 19.5% with no subsequent change. Weight loss significantly correlated with microbial colonization as measured by alkaline phosphatase activity ($r = 0.780$), cellulase activity ($r = 0.613$), heterotrophic CO_2 fixation ($r = 0.835$), and acetate incorporation into microbial lipids ($r = 0.618$). Alkaline phosphatase activity correlated with cellulase activity ($r = 0.889$), and heterotrophic CO_2 fixation correlated with acetate incorporation into lipids ($r = 0.712$). Weight loss after 26 days inversely correlated with the logarithm of the depth of incubation regardless of whether incubation occurred on the sediment surface or in the water column. These findings suggest that a rapid initial period of microbial colonization is driven by nutrients derived from the litter and that the rate of these processes is controlled by a factor(s) inversely related to the logarithm of depth, such as light intensity, primary production, or turbulence.

Decomposition of plant litter in aquatic systems involves leaching and the action of microorganisms and macroinvertebrates (8, 10). Microorganisms mineralize low-molecular-weight components of the litter and produce extracellular enzymes, which degrade macromolecules into smaller units that leach or are mineralized. In addition, colonizing microorganisms increase the nutritional value of the litter and thereby condition it for invertebrate feeding (8).

Toolik Lake is large deep-water lake located approximately 190 km north of the Arctic Circle and 217 km south of Prudhoe Bay, Alaska, in the Trans-Alaska Pipeline Corridor (68° 38′ N; 149° 38′ W). It is oligotrophic, becomes stratified during the summer, and is relatively unimpacted by humans. The shoreline and shallows are dominated by sedges of the genus *Carex*. Great potential exists of *Carex* litter entering the carbon cycle of the lake via terrestrial wash-in and a seasonal dying of emergent plants in the littoral zone.

Various microbial activities have been used to examine the microbial colonization of leaf litter in aquatic systems as a function of time of incubation (1, 9, 16). During the summer of 1978, the weight loss of *Carex* litter at various sites and depths within Toolik Lake was examined as a function of its colonization by microorganisms, measured by microbial activities. Colonization was determined by measuring heterotrophic CO_2 fixation, ^{14}C-labeled acetate incorporation into lipid, alkaline phosphatase activity, and cellulase activity associated with the decomposing litter. After 13 and 26 days of incubation, the degree of weight loss positively correlated with microbial activities and was inversely related to depth of incubation.

MATERIALS AND METHODS

Preparation of litters. Fresh green *Carex* leaves were collected from the margins of Toolik Lake, cut into 5-cm segments, and oven-dried at 65°C. Approximately 300 mg of this dried litter was sewn into 8-by-15-cm bags constructed of fiber glass window screening (1.5-mm mesh). Duplicate bags for both weight loss determination and activity measurements were placed on the sediment surface at four sites within the lake and at three depths in the water column (Fig. 1 and Table 1). Bags were sampled from the sediment surface sites after 13 and 26 days of incubation and from the water column only after 26 days. Abiotic controls consisted of litter-filled screen bags sealed in Whirlpaks (Nasco), which contained 0.1% Merthiolate [ethyl(2-mercaptobenzoato-*S*)mercury sodium salt], and were incubated in the lake. Upon harvesting, the bags were gently washed in lake water and brushed to remove any surface materials, which were generally inconsequential. Weight loss was determined gravimetrically after bags were oven-dried to constant weight. Upon sampling, leaf segments for activity measurements were cut into 1.25- and 2.50-cm segments and stored in filter-sterilized lake water (0.45 μm; Millipore) until used, which was never more than 6 h.

Alkaline phosphatase activity. Five leaf seg-

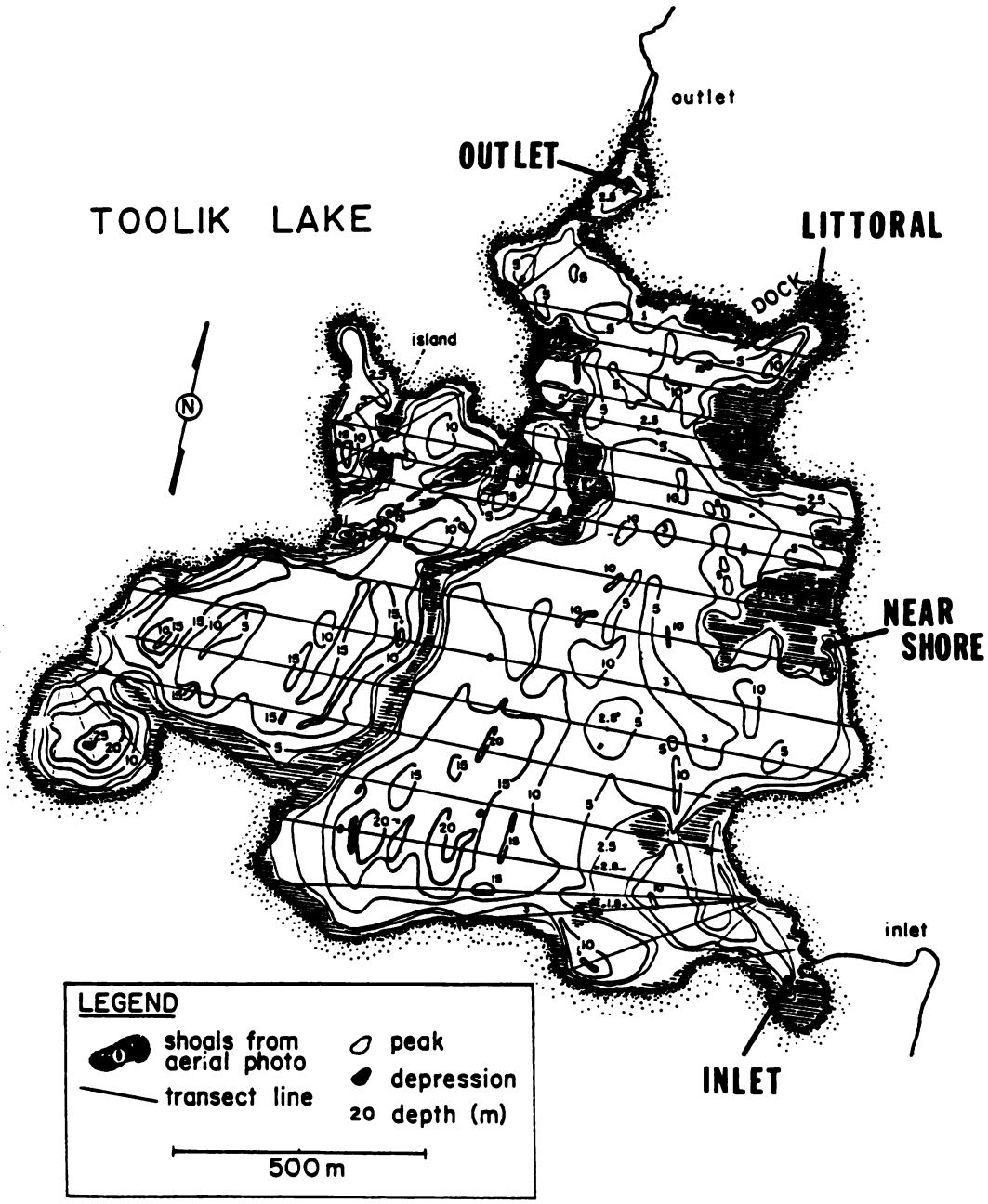

FIG. 1. *A map of Toolik Lake, Alaska, showing the incubation sites used in this study.*

ments were placed in 2 ml of 5 mM *p*-nitrophenyl phosphate (disodium salt) (Sigma) in 25 mM $NaHCO_3$ buffer (pH 9) for 20 min at 20 ± 2°C (1, 9). The reaction was stopped by removal of the segments and addition of 2 ml of 50 mM $NaHCO_3$ buffer (pH 9.0). The samples were immediately read at 410 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb). Activity was expressed as the rate of release (in micromoles) of *p*-nitrophenol from the phosphate ester per hour per 5-cm segment.

Cellulase activity. Cellulase activity was determined by measuring reducing units released from carboxymethylcellulose as described by Petterson and Porath (11). Five leaf segments were placed in 2 ml of 1% carboxymethylcellulose (sodium salt) (Matheson) in acetate buffer (pH 5.0) for 20 to 25 h at 15 ± 1°C. Dinitrosalicylic acid reagent (3 ml) was added to each sample, which was then placed in a boiling-water bath for 15 min. Color was read at 640 nm with a Spectronic 20. Activity was expressed as the rate of production of

TABLE 1. *Incubation sites in Toolik Lake, Alaska*

Site	Depth (m)	Temp range[a] (°C)	Sediment (% organic)	Location[b]
Inlet	1.6–2.0	7.7–14.3	22	Near mouth of inlet stream
Outlet	5.5–6.0	7.1–9.8	20	Near mouth of outlet stream
Nearshore	2.5–2.8	7.5–14.2	22	Distant from inlet and outlet streams, 5 m from shore
Littoral	0.06–0.12	7.8–15.3	68	In *Carex* bed
Water column	0.7	7.8–15.3		Near center of lake
	4.0	7.2–14.0		
	8.0	6.8–7.9		

[a] During study period, 1 to 26 July 1978 (M. C. Miller, personal communication).
[b] See Fig. 1.

reducing units per hour per 5-cm segment.

Dark heterotrophic CO_2 uptake. Five 2.5-cm segments were placed in 10 ml of filter-sterilized lake water contained in 30-ml glass-stoppered wide-mouth bottles, totally covered with black electrical tape with the stoppers covered with aluminum foil, and allowed to preincubate for 2 h. ^{14}C-labeled sodium bicarbonate (4 μCi) was then added to each bottle and incubated for 4 to 5 h at 15 ± 2°C. The segments were removed, rinsed with filter-sterilized lake water, dried, and counted with a scintillation counter (Packard Tri-Carb) in a cocktail consisting of 0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP [1,4-bis-(5-phenyloxazolyl) benzene] in toluene. CO_2 fixation was calculated as described by Vollenweider (15) and was expressed as micrograms of carbon fixed per hour per 5-cm segment. Available CO_2 in the incubation medium was calculated from pH, temperature, and total alkalinity (12). Lugol's iodine-treated controls were used to correct for non-biological uptake of the isotope

^{14}C-labeled acetate incorporation into lipids. Ten 2.5-cm segments were placed in 10 ml of filter-sterilized lake water with 5.26 μCi of sodium [1-^{14}C]-acetate (7.34 μg) contained in 150-ml capped Fleakers (Corning) for approximately 4 h at 15 ± 2°C. Lipids were extracted from the litter as described by Morrison et al. (9), and radioactivity was determined in the lipid fraction. Incorporation was expressed as the rate of [^{14}C]acetate (micrograms) appearing in the lipid fraction per hour per segment. Lugol's iodine-treated controls were used to correct for non-biological uptake of the isotope.

RESULTS

Large diversity existed in the degree of weight loss observed at the various incubation sites after 13 and 26 days (Table 2). No invertebrates were found associated with the litter bags, indicating that this loss was physical or microbiological in origin. The abiotic controls lost 20% of their weight after 13 days with no further appreciable loss after 26 days. Microbial activity associated with the litter increased with time at each site. In addition, microbial activity at the times examined correlated with the degree of decomposition. Microbial activities were expressed on a per-segment rather than per-weight basis to eliminate bias caused by differential weight loss of the samples.

TABLE 2. *Percent weight loss of dried Carex segments incubated at various sites and depths in Toolik Lake, Alaska*[a]

Site	% Wt loss ($\bar{X}$ ± SD[b])	
	13 days	26 days
Inlet	28.50 ± 1.23	45.65 ± 0.92
Outlet	24.15 ± 0.57	27.69 ± 4.51
Nearshore	32.02 ± 2.64	41.82 ± 1.00
Littoral	33.56 ± 11.26	65.01 ± 9.08
Depth series		
0.7 m	—[c]	55.24 ± 0.93
4.0 m	—	47.51 ± 2.67
8.0 m	—	36.97 ± 1.20
Abiotic control[d]	19.58 ± 0.19	19.45 ± 0.94

[a] The experiment was started on 1 July 1978.
[b] SD, Standard deviation.
[c] —, Not done.
[d] 0.1% Merthiolate.

Alkaline phosphatase activity (Fig. 2A) varied at day 13 from 0.220 to 0.48 μmol of *p*-nitrophenol released per h per segment. The lowest activity was observed at the outlet, which showed the least decomposition (24%); the highest activity was at the nearshore site. At 26 days, activity varied between 0.35 and 0.73 μmol of *p*-nitrophenol released per h per segment, with the least decomposed sample (outlet) once again exhibiting the lowest activity. The two most decomposed samples exhibited activities that were relatively high but below the maximum observed. Alkaline phosphatase activity was significantly correlated with percent weight loss ($P \leq 0.005$) (Fig. 2A), but the lower activities observed with the greatest weight losses tend to indicate a leveling and decrease in activity relative to percent weight loss.

Cellulase activity correlated with alkaline phosphatase activity ($P \leq 0.001$) (Table 3) and, to a lesser degree, with the degree of weight loss ($P \leq 0.05$) (Fig. 2B). It ranged from 0.206 to 0.236 reducing units released per h per segment at day 13 and from 0.236 to 0.290 reducing units

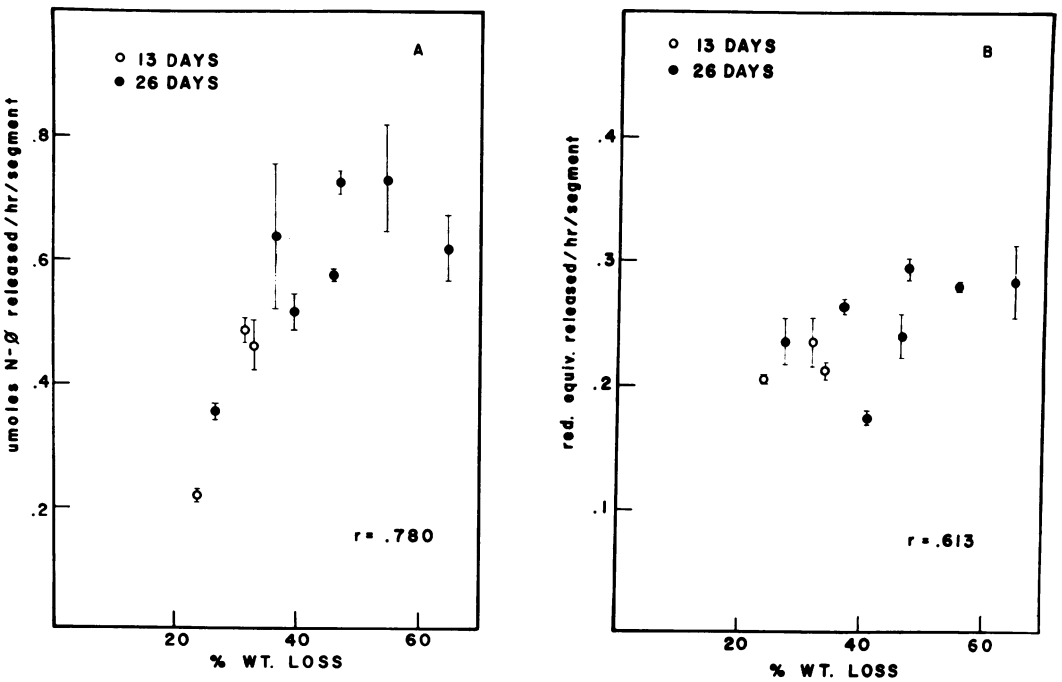

FIG. 2. *Microbial activities on Carex litter as a function of weight loss after incubation for 13 and 26 days in Toolik Lake. (A) Alkaline phosphatase activity; (B) cellulase activity.*

TABLE 3. *Correlation matrix of microbial activities on Carex litter in Toolik Lake after 13 and 26 days*

Activity	Alkaline phosphatase activity	Cellulase activity	Heterotrophic CO_2 fixation	Acetate incorporation
Alkaline phosphatase activity		0.889 ($P \leq 0.001$)	0.602 (NS)[a]	0.573 (NS)
Cellulase activity			0.561 (NS)	0.371 (NS)
Heterotrophic CO_2 fixation				0.712 ($P \leq 0.01$)

[a] NS, Not significant.

released per h per segment at day 26 (Fig. 2B). Once again, samples from the outlet site showed the lowest activities on both dates.

Although not significantly correlated with either cellulase activity or alkaline phosphatase activity, heterotrophic CO_2 fixation and acetate incorporation were significantly correlated with each other ($P \leq 0.01$) (Table 3) and with percent weight loss ($P \leq 0.05$ and $P \leq 0.001$, respectively). Heterotrophic CO_2 uptake varied from 0.0237 to 0.0422 and 0.0348 to 0.0708 μg of C per h per segment on days 13 and 26, respectively (Fig. 3A). On day 26, the highest activity was associated with the most degraded leaf material. Acetate incorporation into lipids ranged between 0.050 and 0.068 and between 0.046 and 0.091 μg/h per segment on day 13 and 26, respectively (Fig. 3B), with the outlet exhibiting the lowest activity on both dates. As with alkaline phosphatase activity, the most degraded samples exhibited lower activities than the maximum, once again indicating a possible leveling pattern and decrease relative to weight loss.

Weight loss showed a decreasing pattern with depth of incubation in the samples incubated in the water column (Fig. 4). This pattern was found, likewise, for samples incubated on the sediment surface. On day 26, the degree of weight loss was significantly ($P \leq 0.01$) related to the logarithm of the depth of incubation regardless of whether incubation occurred on the sediment surface or in the water column.

DISCUSSION

Considering the temperature, nutrient availability, and productivity of Toolik Lake, a surprisingly large amount of decomposition occurred in a relatively short time. The degree of weight loss in this study was higher than observed in other studies (2), which may be accounted for by the use of fresh green litter and use of relatively small amounts of litter in each bag. In most studies, larger amounts of litter were placed in each bag, which may have re-

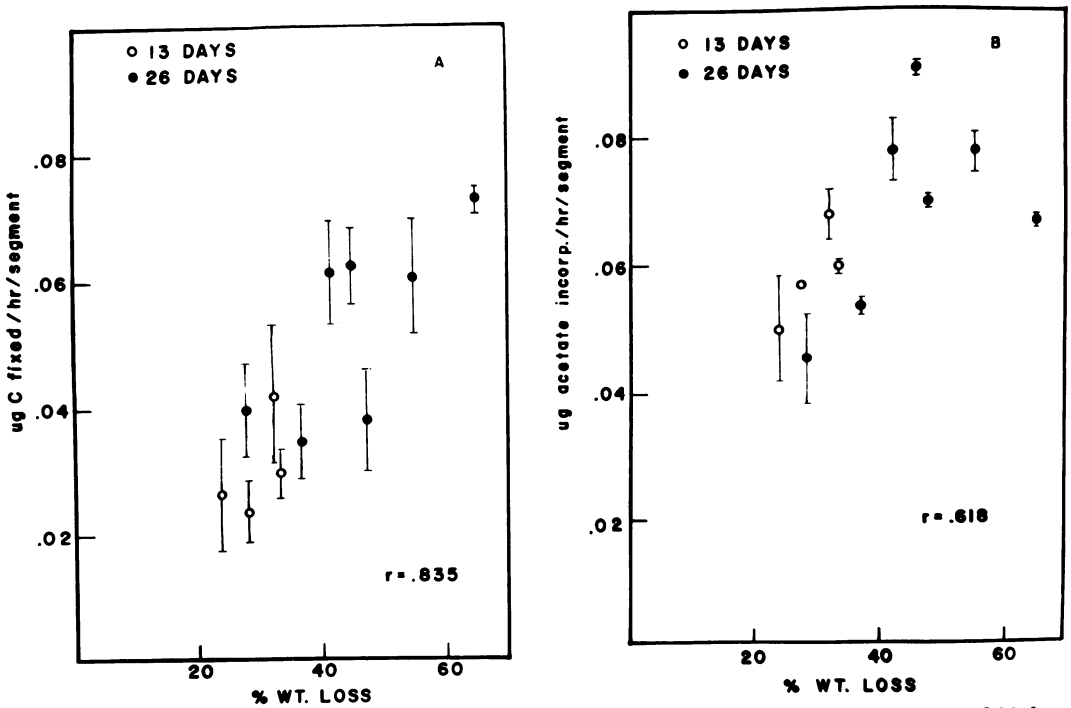

FIG. 3. *Microbial activities on Carex litter as a function of weight loss after incubation for 13 and 26 days in Toolik Lake. (A) Heterotrophic CO_2 fixation. (B) Acetate incorporation into microbial lipids.*

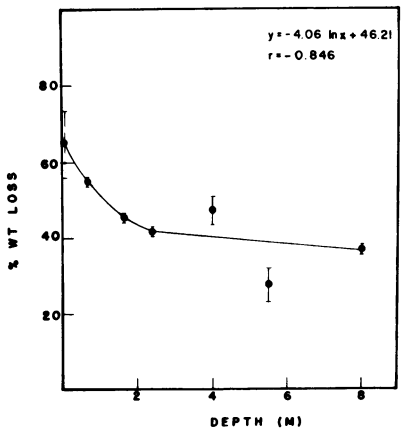

FIG. 4. *The relationship of weight loss by Carex litter in Toolik Lake as a function of depth of incubation.*

sulted in localized nutrient limitation and anaerobic conditions within the bag, accounting for slower rates of decomposition (3, 8).

The large difference in percent weight loss between the samples incubated in the lake and the killed controls, as well as an absence of macroinvertebrates, implicates microorganisms as being largely responsible for the degradation of *Carex* litter in Toolik Lake. It should be recognized that the killed controls in this study may have been underestimates due to protection of the litter from wind and wave action and the presence of diffusion gradients in the smaller volume. Nevertheless, the positive correlations between weight loss and the various microbial activities suggest a functional relationship between decomposition and microbial colonization.

Morrison et al. (9) found that microbial activities associated with plant litter placed in a semitropical estuary followed three different patterns with time: (i) continual increase; (ii) increase to a maximum followed by a rapid decline; and (iii) increase to a plateau, which was maintained. They also found, along with other workers, that acetate incorporation and alkaline phosphatase activities correlate with various other estimates of microbial activity and biomass (7, 9, 14, 16).

The results of this study suggest that the initial rise in activities observed by Morrison et al. (9) may be driven by components of the litter that are easily degraded and utilized by the microorganisms, and that any leveling or decrease of activities may result from the depletion of these easily degraded components, leaving only those more refractory. This contention is further supported by the finding that microbial activities and biomass were much higher on pine

needles than on less degradable, polyvinyl chloride Christmas tree needles incubated in a semitropical estuary (1). It is noteworthy that the alkaline phosphatase activity associated with the pine litter was an order of magnitude greater than that observed with *Carex* litter in this study on a per-weight basis.

The finding that the enzyme activities were not correlated with acetate incorporation into lipids and dark CO_2 fixation, which were correlated to each other, suggests that these two types of measurements examine different aspects of the microbial community which, although related to decomposition, are mutually exclusive of each other. Further studies may reveal information to interpret these various indices with regard to successional changes in the microbial community.

The relationship between percent weight loss and depth indicates that a factor(s) associated with depth plays a role in controlling the rate of decomposition of *Carex* in Toolik Lake. Temperature has previously been shown to have an effect on the decomposition of plant material in freshwater systems. Suberkropp et al. (13) found that short-term weight loss of hickory and oak leaves correlated with mean temperature in Augusta Creek, Mich. Iversen (6) reported a similar relationship for beech leaves in three small Danish streams. Brinson (2) found a correlation between soil-water temperature and the disappearance of pure cellulose sheeting in a North Carolina swamp. In Toolik Lake, maximum temperature declines with depth, as do light intensity and primary production (M. C. Miller, personal communication). The lowest weight loss, and often the lowest microbial activities, occurred at the outlet site. This site was the second deepest, but showed considerably less weight loss than the deepest. Apparently, another factor not associated with depth was operational at this site. Water flowing in the outlet stream itself had somewhat lower mean levels of nitrate (0.23 μg-atom/liter) than in the inlet stream (1.08 μg-atom/liter) and the lake proper (2.40 μg-atom/liter). The mean ammonia level in the outlet (0.68 μg-atom/liter) was lower than that of the inlet (0.95 μg-atom/liter) and lake (0.93 μg-atom/liter) (Vera Alexander, personal communication). In laboratory microcosm studies, enrichment with nitrate or ammonia has resulted in increased rates of leaf decay (5, 8). Degradation of pure-cellulose cotton duck in streams has also been correlated with nitrate levels (4). Temperature and inorganic nutrient levels, therefore, probably are of prime importance in controlling *Carex* decomposition in Toolik Lake. Nevertheless, without further testing, the possible effects of turbulence, light, and primary production cannot be disregarded.

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LITERATURE CITED

1. **Bobbie, R. J., S. J. Morrison, and D. C. White.** 1978. Effects of substrate biodegradability on the mass and activity of the associated estuarine microbiota. Appl. Environ. Microbiol. **35**:179–184.
2. **Brinson, M. A.** 1977. Decomposition and nutrient exchange of litter in an alluvial swamp forest. Ecology **58**: 601–609.
3. **Danell, K., and K. Sjoberg.** 1979. Decomposition of *Carex* and *Equisteum* in a northern Swedish lake: dry weight loss and colonization by macro-invertebrates. J. Ecol. **67**:191–200.
4. **Egglishaw, H. J.** 1972. An experimental study of the breakdown of cellulose in fast-flowing streams. Mem. Ist. Ital. Idrobiol. Suppl. **29**:405–428.
5. **Howarth, R. W., and S. G. Fisher.** 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. Freshwater Biol. **6**:221–228.
6. **Iversen, T. M.** 1975. Disappearance of autumn shed beech leaves placed in bags in small streams. Verh. Internat. Verein. Limnol. **19**:1687–1692.
7. **Jones, J. G.** 1972. Studies on freshwater micro organisms—phosphatase activity in lakes of differing degrees of eutrophication. J. Ecol. **60**:777–791.
8. **Kaushik, N. K., and H. B. N. Hynes.** 1971. The fate of the dead leaves that fall into streams. Arch. Hydrobiol. **68**:465–515.
9. **Morrison, S. J., J. D. King, R. J. Bobbie, R. E. Bechtold, and D. C. White.** 1977. Evidence for microfloral succession on allochthonous plant litter in Apalachicola Bay, Florida, USA. Mar. Biol. **41**:229–240.
10. **Petersen, R. C., and K. W. Cummins.** 1974. Leaf processing in a woodland stream. Freshwater Biol. **4**:343–368.
11. **Petterson, G., and J. Porath.** 1966. A cellulolytic enzyme from *Penicillium notatum*. Methods Enzymol. **8**: 603–607.
12. **Saunders, G. W., F. B. Trama, and R. W. Bachman.** 1962. Evaluation of a modified ^{14}C technique for shipboard estimation of photosynthesis in large lakes. Great Lakes Res. Div. Publ. Ann Arbor. no. 8. University of Michigan.
13. **Suberkropp, K., M. J. Klug, and K. W. Cummins.** 1975. Community processing of leaf litter in woodland streams. Verh. Internat. Verein. Limnol. **19**:1653–1658.
14. **Taga, N., and H. Kobori.** 1978. Phosphatase activity in eutrophic Tokyo Bay. Mar. Biol. **49**:223–229.
15. **Vollenweider, R. A.** 1969. A manual on methods for measuring primary production in aquatic environments. F. A. Davis Co., Philadelphia.
16. **White, D. C., R. J. Bobbie, S. J. Morrison, D. K. Oosterhof, C. W. Taylor, and D. A. Meeter.** 1977. Determination of microbial activity of estuarine detritus by relative rates of lipid biosynthesis. Limnol. Oceanogr. **22**:1089–1099.