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23. MICROBIAL ACTION IN DETRITAL LEAF PROCESSING AND THE EFFECTS OF CHEMICAL PERTURBATION

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ABSTRACT

A laboratory study was conducted to determine the effects of microbial action in detrital leaf processing and to quantify the effects of an anti-microbial agent on microbial functions. Preleached disks from leaves of *Acer saccharum* were incubated in flasks of stream water on a rotary shaker at 20°C for 56 days. Treatments consisted of a control, killed control, and 1.0 and 10.0 mg/l sodium pentachlorophenol (PCP). Leaf disks were periodically measured for changes in dry weight, ash weight, ash-free dry weight (AFDW), and nitrogen. Microbial measurements included respiration, adenosine triphosphate, and ^{14}C acetate incorporation in lipids. Changes in AFDW of leaf disks in the control indicated that microbes processed 21% of leaf material during the 56-day study. The PCP treatments did not differ significantly from the control in loss of

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AFDW from leaf disks. Significant differences were noted among several microbial characteristics, however; this possibly indicates structural differences caused by the PCP treatments. Chemical perturbation could alter the structure of microbial communities and therefore adversely affect leaf processing, invertebrate productivity, and fishery resources.

INTRODUCTION

During the last 15 years a conceptual scheme has been developed concerning the processing of organic matter in small streams (Minshall, 1967; Cummins, 1974). Basic to this sequence of events is the microbial colonization of detrital material to form what is known as the matter–microbial complex (Cummins et al., 1972). This processing unit is an important link in the transfer of fixed solar energy from the terrestrial watershed to the heterotrophic stream community (Fisher and Likens, 1973).

Using detritus both nutritionally and as a site of activity, bacteria and fungi oxidize both dissolved and particulate allochthonous organics (Cummins, 1974). Microbial production increases the net protein content of the decomposing matter (Kaushik and Hynes, 1971). Invertebrates, in turn, shred and scrape the colonized organic matter to derive nutrition (Cummins, 1974). Thus microbial populations provide an improved food source for detritivores, while also reducing the organic load of streams.

The relative importance of microbial populations in processing particulate organic matter, however, warrants further study (Anderson and Sedell, 1979). The contribution of microbial populations is difficult to determine in the presence of invertebrates, epiphytic algae, and physical abrasion, which combine in complex systems of synergistic and antagonistic processes. Therefore we conducted a laboratory study to help corroborate the importance of microbial processing of leaves and to examine trends among parameters known to be associated with detrital leaf processing. In addition, we sought to determine the extent to which an environmental contaminant such as pentachlorophenol (PCP) might disrupt microbial leaf processing within streams. Manufactured at the rate of 50 million lb/yr, PCP is used in the wood-products industry to retard decay of wood and is also used to prevent algal and microbial growth in cooling towers (Cirelli, 1978). The magnitude of production and use confirm the potential for the entry of PCP into aquatic systems.

MATERIALS AND METHODS

Leaves of sugar maple (*Acer saccharum*) were collected at abscission during autumn 1979. The leaves were pressed, lyophilized, and stored frozen. Before experimental treatment began, 6-mm disks were punched from leaves of similar size, shape, and texture. The leaf disks were preleached at 20°C for 36 h in well water to remove readily soluble organics.

Incubation water was obtained on March 23, 1980, from Clifty Creek, a relatively undisturbed oak–hickory watershed overlying limestone bedrock near Hayden township, Maries County, Missouri. Initial water conditions were: temperature, 17°C; conductivity, 305 μmho/cm; pH, 8.0; alkalinity, 135 mg/l as $CaCO_3$; and Kjeldahl nitrogen, 0.73 mg/l as N.

On March 24, 1980, 60 leaf disks and 300 ml of unfiltered stream water were incubated in 500-ml Erlenmeyer flasks on a rotary shaker at 20 ± 2°C in the dark. Treatments consisted of an untreated biotic control, killed control (100 mg/l $HgCl_2$), low PCP (1.0 mg/l Na salt of PCP), and high PCP (10.0 mg/l Na-salt of PCP). Triplicate flasks within each treatment were sampled on days 1, 3, 7, 14, 28, and 56 in the following manner.

Temperature, oxygen, conductivity, pH, and alkalinity were determined by conventional means (American Public Health Association, 1975). Water for determinations of dissolved organic carbon (DOC) was filtered through a 0.2-μm Nucleopore membrane filter, acidified to pH 2 with 1.0 N H_2SO_4, and frozen until analysis. The DOC samples were measured with a Technicon Autoanalyzer II Industrial System DOC Cartridge (Technicon Industrial Systems, 1976).*

Upon sampling, leaf disks were lyophilized for 48 h and frozen until analysis. At the time of analysis, leaf disks were lyophilized for an additional 24 h and then stored in a desiccator. Leaf disks were weighed to the nearest 0.1 μg to determine dry weights, ashed at 550°C, and reweighed to determine ash weights. Ash-free dry weight (AFDW) was determined by subtraction.

Nitrogen concentrations in leaf disks and water were determined colorimetrically after Kjeldahl digestion (Kopp and McKee, 1979).

Adenosine triphosphate (ATP) samples were extracted in cold acid by a method modified from Karl and LaRock (1975). Ten-disk subsamples,

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*Mention of products or brand names does not imply Government or University endorsement.

15-ml H_2O filtrates (0.2-μm Nucleopore membrane), and triplicate spikes and blanks were each immersed in 5 ml of 0.6 N H_2SO_4 at 4°C for 30 min (Knauer and Ayers, 1977). The extract was diluted with 2 ml of MOPS diluent, neutralized with NaOH to pH 7.2, and adjusted to 10 ml in volume with MOPS diluent (E. I. DuPont DeNemours and Co., 1970). The extracts were assayed immediately for ATP content with a DuPont 760 Luminescent Biometer. Recoveries of spiked samples (4.46 ng ATP) averaged 64% during the study; sample values were subsequently corrected to reflect this recovery.

Microbial activity in leaf disks and water was estimated by relative rates of lipid biosynthesis determined by ^{14}C acetate uptake and incorporation into lipids (White et al., 1977). Ten leaf disks, 50 ml of flask water, and 12 μCi [1-^{14}C] of acetate (specific activity, 59 mCi/mmole) were incubated for 2 h in a 125-ml Erlenmeyer flask on a rotary shaker in the dark at 20 ± 2°C. After incubation the water was filtered through a 0.4-μm Nucleopore membrane filter. Disks and filters were subsequently extracted according to White et al. (1977). The chloroform extract was decanted into a scintillation vial and evaporated to dryness. The vials were filled with 10 ml of scintillation fluid (Beckman Fluoralloy dry mix in toluene) and counted in a Beckman Model LS-3133T Scintillation Counter. Recovery was determined by triplicate extraction of spiked ^{14}C stearic acid. Recoveries of spiked samples averaged 94%, and sample values were subsequently corrected for recovery.

Seven days before respiratory measurements, triplicate flasks were stoppered and allowed to incubate on the darkened shaker table. On the seventh day, 500 μl of the flask airspace was removed with a syringe via a septum in the stopper and injected into a gas chromatograph equipped with a thermal conductivity detector. Carbon dioxide was eluted with a stainless steel column (2 m by 0.32 cm ID) packed with Carbosieve "B" (80/100 mesh) maintained at 90°C. Carbon dioxide evolution was calculated from a regression line obtained from CO_2 analysis of standards.

Concentrations of carbon dioxide dissolved in the water of the stoppered flask were calculated from an algorithm of Stumm and Morgan (1970). Total-flask respiration was calculated as CO_2 (evolved) plus CO_2 (dissolved).

Leaf disk samples were periodically removed and examined by scanning electron microscopy for their characteristic microbial flora and degree of colonization. Disks were fixed in 1% osmium tetroxide for 1 h and stored in absolute ethanol at 5°C. Upon analysis, the disks were critical-point dried, sputter coated with gold, and scanned for dominant microflora with a Joel JSM-1 Scanning Electron Microscope.

In analyzing the data, we plotted variables by treatment through time and conducted a one-way analysis of variance of treatment means using orthogonal contrasts (Chew, 1977) on each sampling day. Differences were considered statistically significant at $p \leqslant 0.05$.

RESULTS

Water Chemistry

The water chemistry of the biotic control, low PCP, and high PCP treatments was stable through time, with no significant differences caused by the treatment. The measurements and their ranges were: temperature, 18 to 22°C; conductivity, 260 to 283 μmho/cm; pH, 8.25 to 8.44; dissolved oxygen, 8.2 to 8.7 mg/l; and total alkalinity, 120 to 132 mg/l as $CaCO_3$. The water chemistry in the killed control, however, differed from these treatments in conductivity, pH, and alkalinity caused by the $HgCl_2$. The killed control measurements and their ranges were: temperature, 18 to 22°C; conductivity, 291 to 307 μmho/cm; pH, 8.06 to 8.26; dissolved oxygen, 8.3 to 8.7 mg/l; and total alkalinity, 94 to 124 mg/l as $CaCO_3$. Although most measurements in the killed control were uniform through time, the alkalinity decreased.

Microbial Biomass

Ratios of cellular carbon to ATP are fairly constant for a wide variety of microorganisms (Holm-Hansen and Booth, 1966). Properly employed, the ATP concentration of detrital matter provides a rapid, sensitive estimate of microbial biomass (Bancroft et al., 1976).

ATP-Disks

The ATP concentrations of microbes associated with leaf disks increased from 2.0 to 12.5 ng ATP/disk in the biotic control and low PCP treatments through the course of the experiment (Figure 1). The trend in these treatments amounted to a 525% increase in ATP content of leaf disks during the 8 weeks. These two treatments paralleled each other through time, with no significant differences between them. The high PCP treatment, however, remained significantly lower than the biotic control on every sampling date (Figure 1). On the average, the ATP content of leaf disks in the high PCP treatment was only 38% of that in
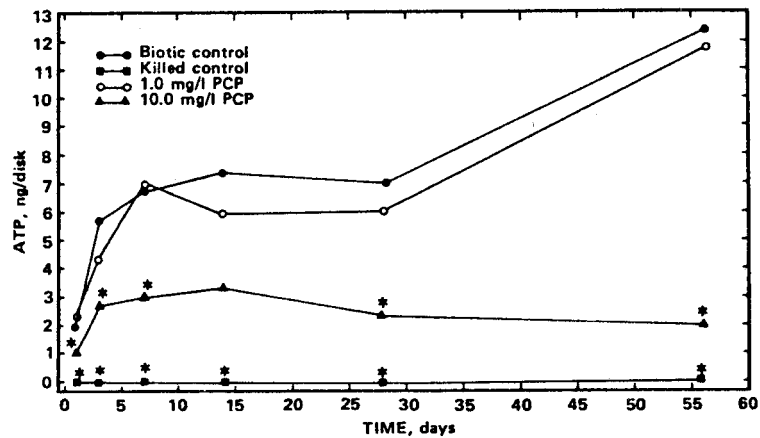
Figure 1. Changes in ATP content of leaf disks over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leqslant 0.05$.
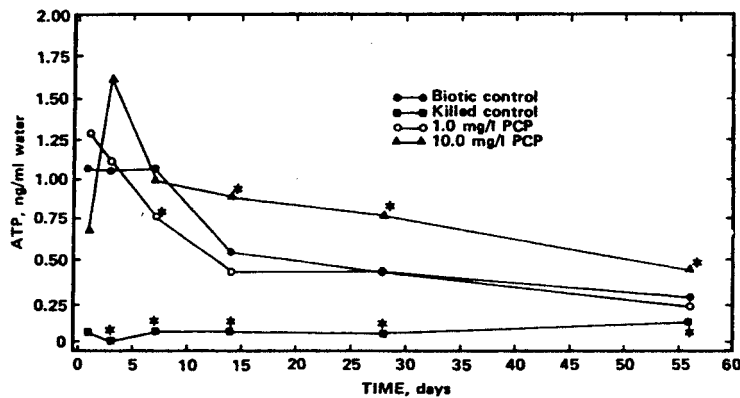

Figure 2. Changes in ATP content of water over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leqslant 0.05$.

the biotic control. The ATP concentration of leaf disks in the killed control remained at minimally detectable levels (Figure 1).

ATP-Water

The ATP concentration of water and its suspended microbes decreased through time in the biotic control, low PCP, and high PCP treatments (Figure 2). The ATP in biotic control and low PCP treatments decreased nearly 65% in 56 days. In general, these two treatments did not differ
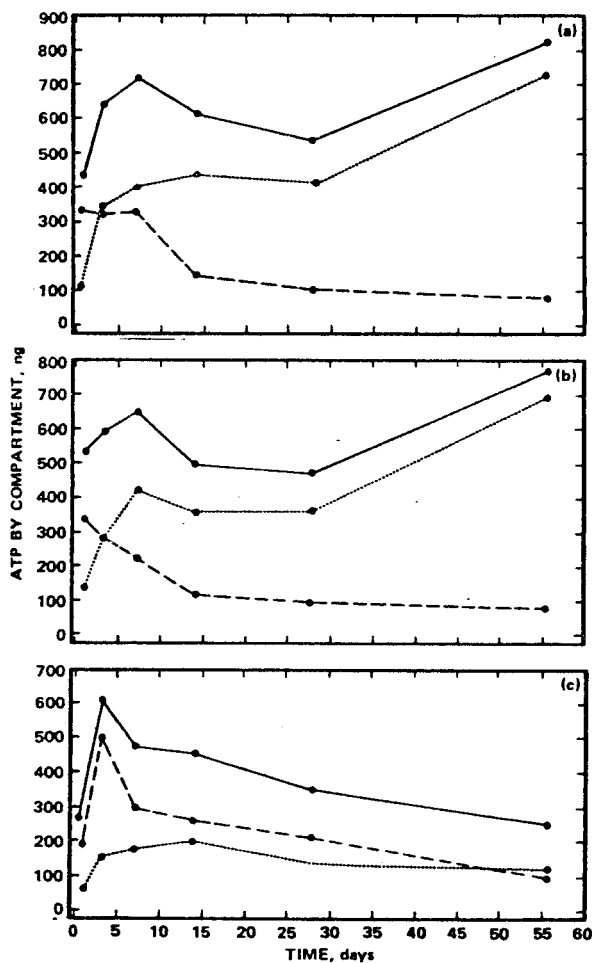
Figure 3. Changes in ATP of various flask compartments over time for (a) biotic control, (b) low PCP (1.0 mg/l), and (c) high PCP treatments (10.0 mg/l). Solid lines denote total flask ATP; dotted lines, ATP associated with 60 leaf disks; and dashed lines, ATP associated with 300 ml of flask water.

ment can be used as a relative measure of heterotrophic microbial activity (White et al., 1977).

^{14}C Lipid-Disks

The uptake and incorporation of ^{14}C acetate into microbial lipids associated with leaf disks increased rapidly in the biotic control during the
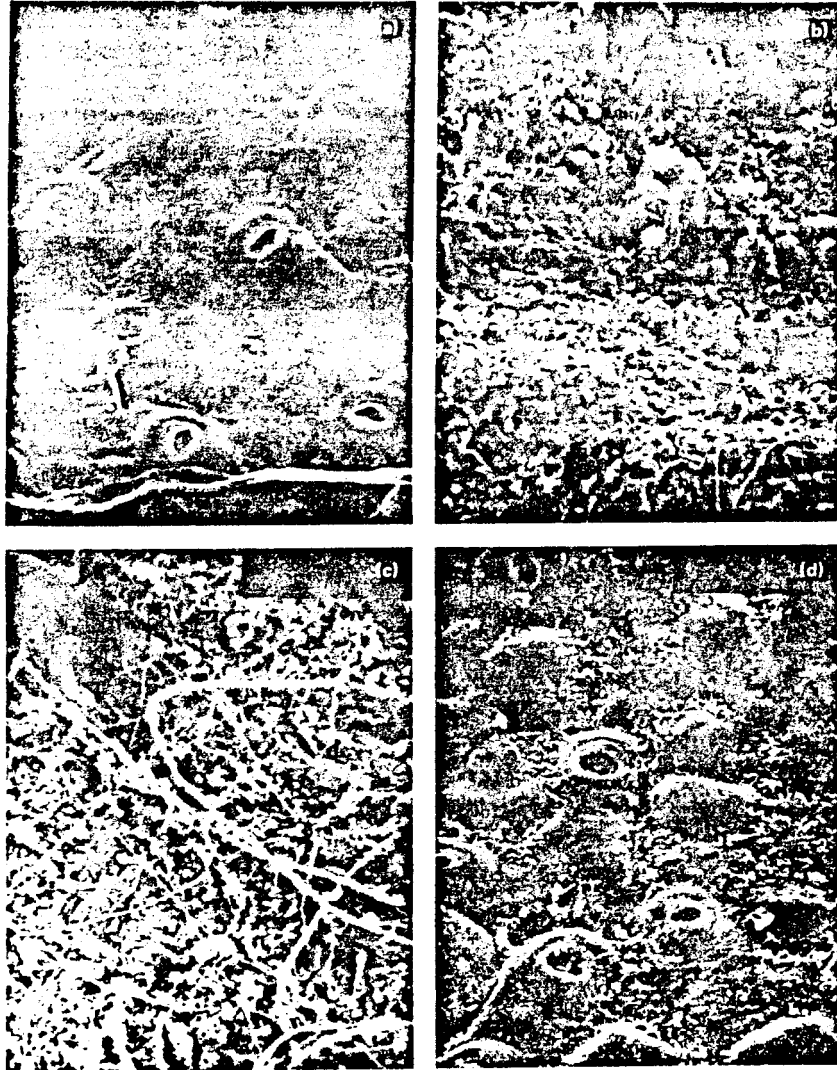
Figure 4. Scanning electron micrographs of the ventral surfaces of leaf disks. (a) Day 0. (b) Day 10, biotic control. (c) Day 20, biotic control. (d) Day 20, killed control. All photos were magnified 1000X.
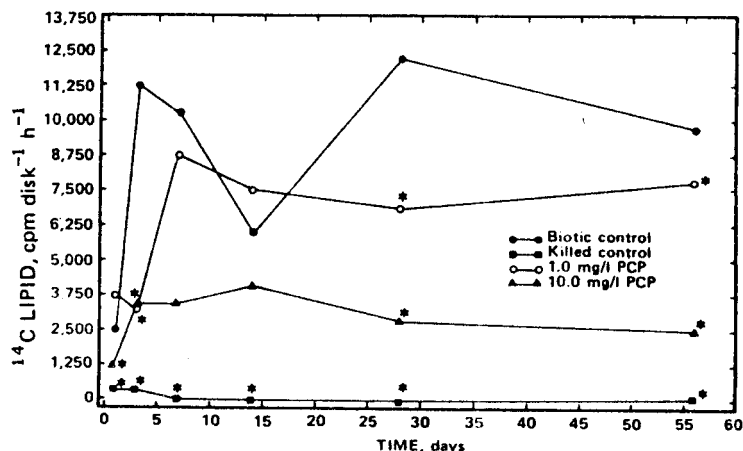
Figure 5. Changes in rates of ^{14}C lipid synthesis in the microbial community associated with leaf disks. Plots represent ^{14}C lipid synthesis over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leq 0.05$.

first three days of the study (Figure 5). The rate decreased in the biotic control on days 7 and 14 but increased at days 28 and 56 to about 11,000 cpm disk^{-1} h^{-1}. The rates of ^{14}C lipid synthesis by disk microbes in the low and high PCP treatments increased during the first 7 days of the study (8750 and 3400 cpm disk^{-1} h^{-1}, respectively) (Figure 5). The rates remained stable through day 56 but were significantly lower than in the biotic control on days 28 and 56. On day 56 the rates of ^{14}C lipid synthesis were 20% less in the low PCP treatment and nearly 70% less in the high PCP treatment than in the biotic control. Leaf disks in the killed control did not exhibit any appreciable uptake and incorporation of ^{14}C acetate during the experiment (Figure 5).

^{14}C Lipid-Water

Uptake and incorporation of ^{14}C acetate into lipids by the suspended microbial populations declined through time in the biotic control, low PCP, and high PCP treatments (Figure 6). Rates in the biotic control and low PCP treatments declined 80% in 56 days to 700 cpm ml^{-1} water h^{-1}. In contrast, the rate in the high PCP treatment declined only 35% in 56 days to approximately 1500 cpm ml^{-1} water h^{-1} (Figure 6). The day-56 value was significantly greater in the high PCP treatment than in the biotic control and corresponds to the significantly greater ATP concentration (water) in the suspended microbial community. There was little
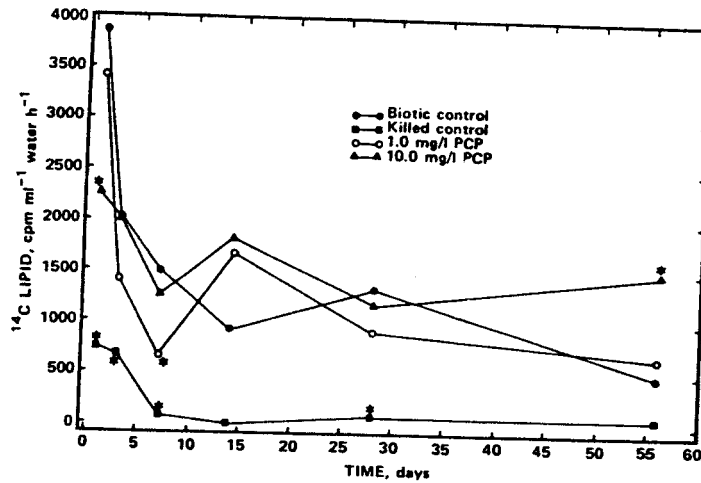
Figure 6. Changes in rates of ^{14}C lipid synthesis by the suspended microbial community. Plots represent ^{14}C lipid synthesis over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leqslant 0.05$.

incorporation of ^{14}C acetate by suspended microbes in the killed control (Figure 6).

^{14}C Lipid-Total

By partitioning the ^{14}C lipid into the total associated with the 60 leaf disks, the total associated with the 300 ml water, and total flask ^{14}C lipid, we can demonstrate that the biotic control, low PCP, and high PCP treatments differed in the distribution of microbial activity. Microbial activity in the biotic control and low PCP treatments shifted from the water to the disk locus through time (Figure 7). On day 56 approximately two-thirds of the total activity was associated with leaf disks in these two treatments. In the low PCP treatment, however, ^{14}C lipid synthesis by disk microbes was significantly lower on days 28 and 56 (Figure 5). Furthermore, the trends in ^{14}C lipid synthesis in the low PCP treatment did not parallel fluctuations within the biotic control (Figure 7). The fluctuations in the biotic control data may indicate succession in the microbial community (White et al., 1977). Similar, parallel fluctuations were not observed in the low PCP treatment; this may indicate that chemical perturbation prevented such succession.

The distribution of microbial activity in the high PCP treatment also seemed to differ from that in the biotic control. Primarily disk associated
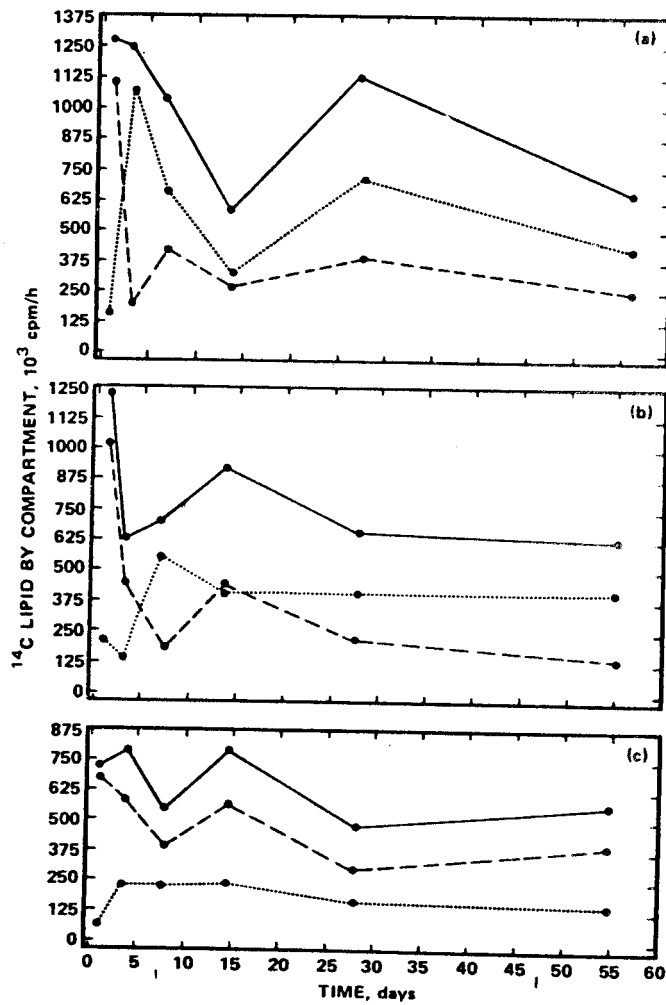
Figure 7. Changes in rates of ^{14}C lipid synthesis in the various flask compartments over time for (a) the biotic control, (b) low PCP (1.0 mg/l), and (c) high PCP (10.0 mg/l) treatments. Solid lines denote total flask synthesis; dotted lines, synthesis associated with 60 leaf disks; and dashed lines, synthesis associated with 300 ml flask water.

in the biotic control, ^{14}C lipid synthesis was mostly in the aqueous compartment of the high PCP treatment (Figure 7). This trend was similar to that shown for ATP and suggests that the high PCP treatment altered the production, distribution, or succession of the microbial community.
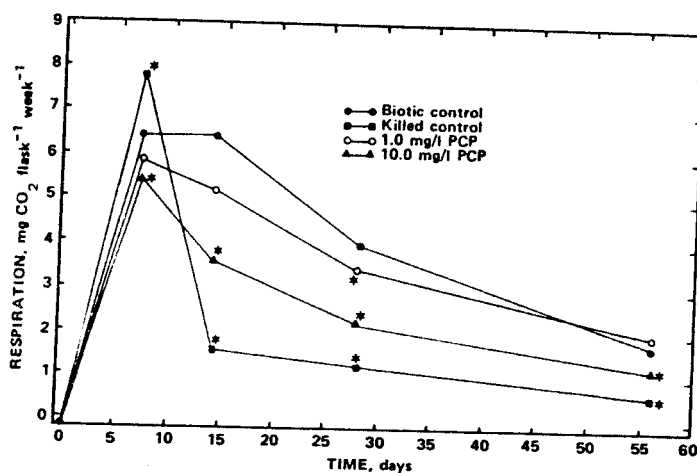
Figure 8. Changes in whole-flask respiration over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leq 0.05$.

Respiration

Since heterotrophic organisms use fixed organic carbon as their primary energy source, and carbon that is not incorporated or egested is liberated as respiratory CO_2, respiration levels can provide a relative estimate of rates of leaf decomposition caused by microbial activity.

The respiration rate declined among all treatments through time (Figure 8). In the biotic control and low PCP treatments, it declined from about 6 mg CO_2 flask^{-1} week^{-1} at week 1 to about 2 mg CO_2 flask^{-1} week^{-1} by week 8. This amounted to a 66% decrease over the course of the study. On most sampling days these two treatments were not significantly different from one another. Respiration in the high PCP treatment decreased 80% in 56 days and was significantly lower than that in the biotic control on each sampling day (Figure 8). The respiration data in the high PCP treatment supported the ATP and ^{14}C lipid synthesis results in indicating that the high PCP treatment may have altered the function as well as the structure of the microbial community. Evolution of CO_2 was also detected in the killed control (Figure 8). Because all other variables indicated that these flasks were sterile, we assumed that the CO_2 evolved in the killed control was probably chemically liberated by the presence of $HgCl_2$.

The decline in respiratory rates during the study may have been caused by depletion of labile organic carbon (Cummins et al., 1972; Wetzel and Manny, 1972). This hypothesis is reinforced by the dissolved organic
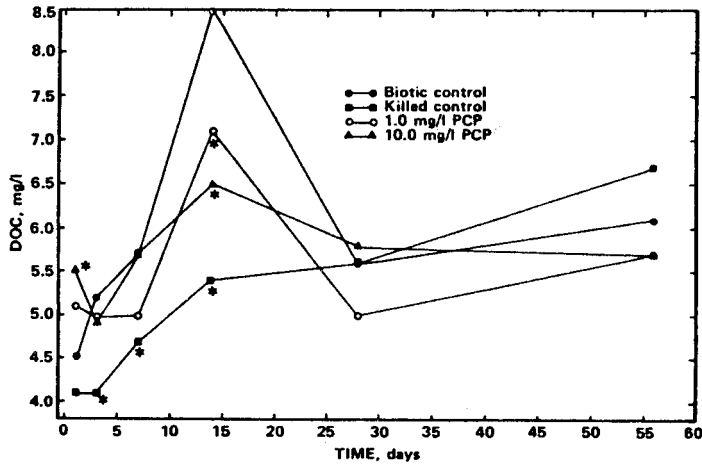
Figure 9. Changes in dissolved organic carbon (DOC) concentrations over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leqslant 0.05$.
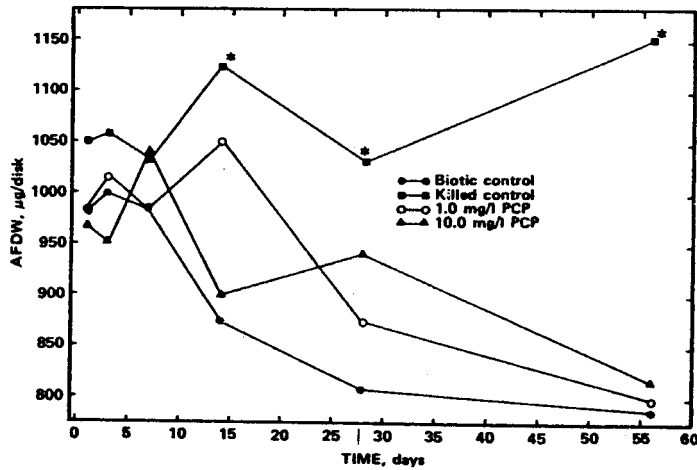

Figure 10. Changes in ash-free dry weight (AFDW) of leaf disks over time by treatment. The asterisk (*) indicates significantly different from biotic control at $p \leqslant 0.05$.

carbon (DOC) data (Figure 9). Respiration was highest on days 7 and 14, the same days on which the DOC levels were highest. Both respiration and DOC declined during the rest of the study as the more readily metabolized elements of the carbon pool were consumed (Figures 8 and 9). Because these were whole-flask respiration estimates, it is not known whether the carbon was oxidized from particulate or dissolved sources.

Processing

Changes in the ash-free dry weight (AFDW) of detrital material are often used as a measure of processing. The AFDW of leaf disks decreased through time in the biotic control, low PCP, and high PCP treatments by 213, 193, and 176 μg/disk, respectively (Figure 10). This decrease amounted to 18 to 21% of AFDW of leaf disks in these treatments. Neither of the PCP treatments were significantly different from the biotic control on any sampling date. Leaf disks in the killed control treatment actually increased in AFDW by over 15% (Figure 10). This increase may have been caused by adsorption (high dry weight measurement) and subsequent volatilization (low ash weight measurement) of mercury during the ashing procedure.

Although the respiration data indicated significant differences in processing between the biotic control and high PCP treatments, AFDW of leaf disks did not. We attributed this in part to the inherent variability in the leaf disks, which may have hindered detection of significant differences in processing. This variability may imply that AFDW of leaf disks is not a highly sensitive indicator of chemical perturbation of leaf processing. In addition, microbes may contribute to loss of organic carbon from leaves in ways other than metabolic oxidation to CO_2. For instance, microbial activity may contribute to further carbon losses from detritus by micro-fragmentation or a form of biotic leaching. Under certain conditions, leaf leachates can form flocculent precipitates with divalent cations that abiotically remove DOC from the water column (Lush and Hynes, 1973). We did not have the analytical capabilities to analyze small particulate organic matter. Lack of information concerning this carbon pool may have caused some error in our processing measurements and may explain why we did not detect significant differences in AFDW of leaf disks in the high PCP treatment.

Nitrogen

The nitrogen concentration of leaf litter is known to increase during microbial decomposition as a result of two processes—an increase in protein as a result of microbial growth on the leaf surface and a simultaneous decrease in organic carbon caused by microbial respiration (Howarth and Fisher, 1976). An increasing nitrogen concentration in detritus indicates that the decomposing matter is increasing in value as an invertebrate food source (Kaushik and Hynes, 1971).

In our 56-day study, the nitrogen concentration of leaf disks increased 18 to 30% in the biotic control, low PCP, and high PCP treatments
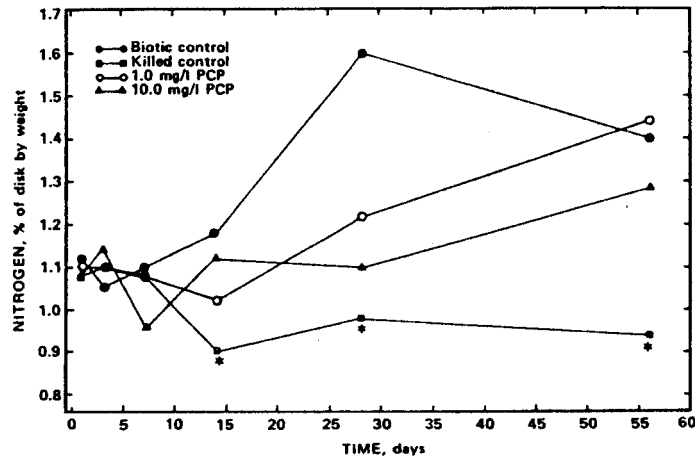
Figure 11. Changes in nitrogen concentration of leaf disks over time by treatment. Nitrogen values are expressed as a percent of disk weight. The asterisk (*) indicates significantly different from biotic control at $p \leqslant 0.05$.

(Figure 11). The two PCP treatments were not significantly different from the biotic control on any sampling day. Nitrogen concentrations of leaf disks in the killed control decreased by 14% in 56 days. This trend may be a result of the leaching of soluble organic nitrogen or may represent a mathematical artifact of the observed increase in dry weight of leaf disks in the killed control. Nitrogen concentrations of leaf disks were significantly lower in the killed control than in the biotic control on days 14, 28, and 56 (Figure 11) because the killed control lacked a microbial community.

The ATP content of leaf disks indicated that microbial biomass associated with leaf disks was significantly lower in the high PCP treatment than in the biotic control (Figure 1). This significant difference was not reflected in the nitrogen concentration of leaf disks because of two inherent problems. First, the variability in weights of leaf disks may have hindered precise calculation of nitrogen concentration. Second, the leaf disks were the major source of nitrogen in the experimental flasks. As microbial protein developed on the leaf disks, nitrogen was merely translocated from the disk to the microbes; thus the disk-microbe complex in the biotic control and PCP treatments contained similar absolute measurements of nitrogen. Although the nitrogen may have been distributed differently between the leaf disks and the microbes in these treatments, it was not discernible in the nitrogen concentration of the disk-microbe complex.

DISCUSSION

Microbial Processing of Leaf Disks

Leaf disks lost 21% of AFDW in the biotic control over the course of the experiment. Leaf processing is known to be the result of four physical and biological factors: (1) abiotic leaching, (2) physical abrasion, (3) microbial metabolism, and (4) invertebrate feeding activity (Cummins, 1974). Invertebrates were intentionally excluded from our system. Lack of changes in ash weights through time indicated that physical abrasion was not a factor in weight loss, and, since leaf disks did not decrease in dry weight during the first 7 days of the experiment, little abiotic leaching occurred. Therefore we attributed the majority of processing to microbial metabolism of leaf disk carbon.

Our estimate of 21% microbial processing of leaves is similar to a 14% estimate obtained with maple leaf disks in a recirculating laboratory stream maintained at 18°C for 5 weeks (Howarth and Fisher, 1976) and to a 24% processing estimate from a laboratory study with unleached elm leaves incubated in streamwater at 21°C for 56 days (Hynes et al., 1974).

There are relatively few estimates of rates of microbial processing of particulate matter in natural streams. Sedell et al. (1975) indicated that leaves decayed slowly by microbial metabolism, but that decomposition increased dramatically after invertebrates entered the leaf packs. These investigators measured over 50% decay of red alder, vine maple, big-leaf maple, and conifer leaves in less than 70 days in a Cascade Mountain stream and attributed this rapid processing to invertebrate feeding. This high percentage suggests that invertebrates mediate most of the leaf decomposition in streams.

However, we cannot ignore the importance of microbial populations in processing leaves in streams. Invertebrates are known to prefer leaves that have been adequately conditioned by microbes (Boling et al., 1975) and select leaves that are most rapidly colonized by microbes (Sedell et al., 1975). Thus microbes play a major role in invertebrate processing activity. Invertebrate activity in itself may increase the ability of microbes to decompose particulate organics. By feeding action, invertebrates reduce the particle size of organic matter; this in turn increases the total surface area available to microbial colonization and metabolism (Cummins, 1974). By excluding invertebrates, we may have reduced the particle-size diversity of organic matter available to microbial decomposition. This reduction may result in an underestimate of the ability of microbial populations to oxidize particulate organic matter. We further recognize that our experimental system may have been nutrient limited; this would

lead to a conservative estimate of microbial processing of leaves. Other researchers have demonstrated increased microbial leaf processing after nutrient enrichment (Howarth and Fisher, 1976; Hynes et al., 1974), and recent work in our laboratory has demonstrated similar results.

We believe that these results have confirmed that microbes can process particulate organic materials to a significant extent. Other research has shown that bacteria are the principal processors of dissolved organic matter derived from terrestrial inputs, leaching, and invertebrate activities (Cummins et al., 1972; Lock and Hynes, 1976). Thus it is evident that microbes probably serve a substantial purpose in processing organic matter within streams. The functional diversity of microbial and invertebrate activity probably plays a significant role in reducing the organic load of streams, thus maximizing heterotrophic production and protecting downstream aquatic resources from organic enrichment.

Potential for Perturbation

Data for the killed control indicated that, in extreme instances, chemical perturbation could completely disrupt microbial leaf processing. The PCP treatments did not significantly affect processing, as judged by the loss of AFDW from leaf disks. Microbial parameters indicated, however, that chemical perturbation by PCP could affect the biomass, distribution, or succession of the microbial community.

Such changes in the microbial community could conceivably disrupt the processing of organic matter in streams. Suberkropp and Klug (1976) determined that fungi are the dominant microbes involved in processing leaf material and that bacteria mainly process fungal excretions and dissolved organic leachates. In addition, Kostalos (1980) recently demonstrated that fungi associated with detritus may be the primary source of nutrition for adult amphipods and other shredding and scraping invertebrates. Therefore a structural change in the microbial community that adversely affects fungal communities could alter not only fungal decomposition of leaves but also invertebrate nutrition and production. A decrease in invertebrate production could eventually have serious fishery implications as well.

We tested only one compound; other types of compounds, which could affect aquatic microbial communities in different ways, should be investigated. Of further interest is the toxicity of environmental contaminants to invertebrate communities. We believe that chemical perturbation might be most damaging at this level since invertebrates are frequently quite sensitive to contaminants and may not be as resilient as microbes. Reduction in invertebrate numbers or diversity could have a multitude of effects on the processing of organic matter and on fishery resources.

ACKNOWLEDGMENTS

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