Effects of Inorganic Nutrients on Microbial Leaf Decomposition and Mitigation of Chemical Perturbation

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ABSTRACT

A laboratory study was conducted to determine (1) the effects of inorganic nitrogen and phosphorus enrichment on microbial leaf processing, and (2) whether inorganic nutrient enrichment would mitigate the effects of chemical perturbation of microbial leaf processing. Pre-leached disks from leaves of Acer saccharum were incubated in flasks of unfiltered stream water at 20°C for 56 days. Treatments consisted of a control, killed control (100.0 mg/l $HgCl_2$), nitrogen (3.0 mg/l NO_3-N), phosphorus (0.1 mg/l PO_4-P), nitrogen + phosphorus (3.0 mg/l NO_3-N + 0.1 mg/l PO_4-P), pentachlorophenol (10.0 mg/l Na-salt of pentachlorophenol), and pentachlorophenol + nitrogen + phosphorus (10.0 mg/l Na-salt of pentachlorophenol + 3.0 mg/l NO_3-N + 0.1 mg/l PO_4-P). Leaf disks in the control treatment lost 31% of ash-free dry weight in 56 days. Leaf decomposition, microbial biomass, and microbial activity increased in all treatments enriched with nitrogen. However, there were no increases in the treatment receiving only phosphorus. This implied that microbial leaf processing in certain aquatic systems may be nitrogen limited. Chemical perturbation by pentachlorophenol significantly reduced biomass and activity of microbes associated with leaves. However, nitrogen + phosphorus enrichment mitigated the effects of chemical perturbation.

INTRODUCTION

Most energy inputs into small woodland streams are in the form of allochthonous organic matter derived from the surrounding watershed (Teal 1957, Minshall 1967, Fisher and Likens 1973). Some of this organic matter enters the stream throughout the year as leaf litter. The energy contained in allochthonous leaves is subsequently transferred to the heterotrophic stream community through a series of abiotic and biotic processing steps (Cummins 1974). An important step in the biotic processing sequence is the colonization and decomposition of organic material by aquatic microbes. Microbes are known to promote leaf decomposition via direct metabolism of the carbon substrate as well as by inviting the macerating activity of aquatic invertebrates (Minshall 1967, Triska 1970, Cummins 1974). In addition, the presence of microbial protein increases the value of detritus as an invertebrate food source (Triska 1970, Kaushik and Hynes 1971, Hynes et al. 1974). Thus, microbial leaf processing is of considerable functional importance to the stream ecosystem.

Recently, concern has arisen concerning man's influence on such vital ecosystem processes. For example, common forestry (Likens et al. 1970, Vitousek et al. 1979) and agricultural (Omernik 1977)

practices have been shown to accelerate losses of inorganic nutrients from terrestrial watersheds. Since terrestrial losses represent the inputs to the stream ecosystem, basic understanding is needed concerning the effects of nutrients on stream processes (Elwood et al. 1981). Also, the potential for chemical perturbation of small woodland streams is quite apparent, since forestry and agricultural industries are becoming increasingly dependent on synthetic chemicals for biocidal uses (defoliants, selective herbicides, insecticides, and fungicides). One such biocide is pentachlorophenol (PCP); manufactured at over 50 million pounds per year in the U.S. in 1974, PCP is commonly used to retard wood-rot and mildew in lumber products and to prevent algal growth in cooling towers of power plants (Cirelli 1978). Pentachlorophenol is toxic to a wide variety of aquatic organisms (Cowell and Anderson 1979), has caused extensive damage to aquatic ecosystems (Matida et al. 1970, Pierce and Victor 1978), can persist for up to two years as residues in sediments and leaf litter (Pierce and Victor 1978), and can alter both structural and functional characteristics of aquatic microbial communities associated with detrital leaves (Fairchild et al. 1983). Thus, accidental releases of PCP into aquatic situations are potentially hazardous. Comparatively little is known, however, about the relative toxicity of such contaminants when they enter aquatic ecosystems in concurrence with inorganic nutrient enrichment following deforestation or tillage. Therefore, this study was conducted to determine 1) the effects of N and P enrichment on microbial leaf decomposition and 2) whether N+P enrichment could mitigate the effects of chemical perturbation of microbial leaf decomposition.

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, 6-mm disks were punched from leaves of similar size, shape, and texture and pooled to minimize variability. Before use, the leaf disks were preleached in well water at 20°C for 36 hours to remove readily soluble organics.

Incubation water (110 µg/l Kjeldahl nitrogen, 27 µg/l total phosphorus) was obtained on June 12, 1980, from Clifty Creek, an undisturbed oak-hickory watershed overlying limestone bedrock near Hayden, Maries County, Missouri. On June 13, 1980, a series of 500-ml Erlenmeyer flasks containing 300 ml unfiltered stream water and 60 leaf disks were incubated on a rotary shaker at 20 ± 2°C in the dark. Treatments consisted of an untreated biotic control, a sterile control (100 mg/l $HgCl_2$), N enrichment (3.0 mg/l NO_3-N), P enrichment (0.1 mg/l PO_4-P), N+P enrichment (3.0 mg/l NO_3-N + 0.1 mg/l PO_4-P), PCP (10.0 mg/l PCP, Na-salt), and PCP+N+P (10.0 mg/l PCP, Na-salt + 3.0 mg/l NO_3-N + 0.1 mg/l PO_4-P). The treatment levels of nitrogen and phosphorus lay within the nationwide averages for 100% forested (0.57 mg/l N and 0.02 mg/l P) and 100% urbanized (3.69 mg/l N and 0.13 mg/l P) watersheds (Omnerik 1977). Pentachlorophenol was obtained from Dow Chemical Company (Dowcide EC-7, 88% purity). The treatment level for PCP was based on data from a previous study (Fairchild et al. 1983). Triplicate flasks within each treatment were sampled on days 14 and 56. The P treatment was only sampled on day 56.

Temperature (20 ± 2°C), oxygen (near saturation), pH (8.09-8.50), and total alkalinity (189-239 mg/l as $CaCO_3$) were determined on days 0, 14, and 56 (American Public Health Association 1975) and remained

relatively constant.

Water for dissolved organic carbon (DOC) analysis was filtered through a 0.2- μm membrane filter, acidified to pH 2 with H_2SO_4, and frozen until analysis. Samples were measured with a Technicon Autoanalyzer II Industrial System DOC Cartridge (Technicon Industrial Systems, Tarrytown, N.Y.).

Leaf disk samples were lyophilized for 48 hours and frozen until analyzed. Leaf disks were weighed to determine dry weights and then ashed at 550°C to determine ash weights. Ash-free dry weight (AFDW) was determined by subtraction.

Kjeldahl nitrogen and total phosphorus concentrations of leaf disks and water were determined colorimetrically after acid digestion (Kopp and McKee 1979). Phosphorus content of water, however, was usually below the limit of detection (5 ug/l) and hence was not reported.

Microbial biomass associated with leaf disks and water was estimated by determining relative levels of adenosine triphosphate (ATP). The ATP samples were extracted in cold acid by the following method modified from Karl and LaRock (1975). Ten-disk subsamples and 10-ml H_2O filtration residues (0.2-μm membrane) from each flask were immersed in separate 5 ml aliquots of 0.6 N H_2SO_4 at 4°C for 30 minutes. The extracts were diluted with 2 ml MOPS diluent, titrated with NaOH to pH 7.2, and adjusted to volume with MOPS diluent (Dupont 760 Luminescent Biometer Instruction Manual, E.I. DuPont DeNemours and Co., Wilmington, Del.). The extracts were assayed immediately for ATP content with a DuPont 760 Luminescent Biometer. Recoveries from triplicate spikes (8.96 ng ATP) averaged 73% during the study; all sample values were corrected for losses.

Heterotrophic activity of microbes associated with 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 flask water, and 12 μCi (1-^{14}C) acetate (Sp. act. 59 mCi/mmole) were incubated for 2 hours 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 membrane filter. Disks and filter membranes were subsequently extracted and measured according to White et al. (1977). Recovery was determined by triplicate extraction of spiked ^{14}C stearic acid. Recoveries from spiked samples averaged 97% and sample values were corrected for recovery.

Seven days prior to respiratory measurements, triplicate flasks were stoppered and allowed to incubate on the darkened shaker table at 20 ± 2°C. 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 Nuclear Chicago Model 5341 Gas Chromatograph equipped with a thermal conductivity detector. Carbon dioxide was eluted with a stainless steel column (2 m x 0.32 cm ID) packed with Carbosieve "B" (80/100 mesh) maintained at 90°C. Concentrations of carbon dioxide dissolved in the water of the stoppered flask was calculated from an algorithm of Stumm and Morgan (1970). Total-flask respiration was calculated as CO_2 (evolved) plus CO_2 (dissolved).

Statistical analysis consisted of a one-way analysis of variance of treatment means on each day using planned orthogonal comparisons (Chew 1977) at the $p \leq .05$ level.

RESULTS AND DISCUSSION

Microbial Leaf Processing and the Effects of N and P Enrichment

Few temporal changes were noted among leaf processing parameters in the sterile treatment during the 56-day study (Tables 1 and 2). Measurements of ATP and ^{14}C lipid synthesis remained near the minimal limit of detection, indicating that this treatment lacked a biological community. Some carbon dioxide evolution was measured in the sterile control. However, this carbon dioxide was probably liberated through physical-chemical processes. The leaf disks used in this study had been preleached to remove readily soluble compounds. Thus few temporal changes were noted in the nitrogen or phosphorus content of leaf disks or water in the sterile treatment. The AFDW (i.e., organic content) of leaf disks actually increased in the sterile treatment during the study (Figure 1), perhaps due to sorption of mercury on leaf surfaces during incubation (high dry weight measurement) and subsequent volatilization upon ashing (low ash weight measurement).

In contrast to the sterile treatment, many chemical and biological changes were observed in the control treatment between days 14 and 56. Measurements of ATP, ^{14}C lipid synthesis, and respiration were significantly greater in the control compared to the sterile treatment on days 14 and 56 (Table 1), indicating that the control contained on active biological community.

Leaf disks in the control treatment lost 31% of AFDW in 56 days (Figure 1). Leaf decomposition in streams is known to result from four physical and biological factors: 1) abiotic leaching, 2) physical abrasion, 3) invertebrate feeding activity, and 4) microbial metabolism (Cummins 1974). The experimental design of this study minimized the effect of the first three factors, therefore we feel that microbial metabolism accounted for most of the leaf processing observed. Our 31% processing measurement agrees with the results of other, similar laboratory studies (Hynes et al. 1974, Howarth and Fisher 1976, Fairchild et al. 1983) and indicates that the microbial community alone can play a significant role in detrital leaf decomposition.

Microbial processing also resulted in temporal increases in the nitrogen and phosphorus content of leaf disks in the control between days 14 and 56 due to the development of microbial protein on the leaf disk surface (Table 2). These increases were noted in both absolute quantity (per disk) and percentage (by weight) of nitrogen and phosphorus. Although the nitrogen content of the leaf disks increased between days 14 and 56, a concommitant decrease was not noted in the nitrogen content of water in the control treatment (Table 2) indicating that the absolute quantity of nitrogen (on a total flask basis) increased in the control treatment between days 14 and 56. A similar nitrogen budget calculated for the sterile treatment did not reveal this increase; this suggests that biological nitrogen fixation may have occurred in the control treatment. Howarth and Fisher (1976) demonstrated that microorganisms associated with detrital leaves have nitrogen fixing abilities, and we believe that our results further support their findings.

The addition of inorganic phosphorus alone had little effect on microbial leaf processing. Leaf disks in the P treatment lost 20% of AFDW in 56 days (Figure 1). However, neither this nor any other measurement in the P treatment was significantly different from biotic control values (Tables 1 and 2).

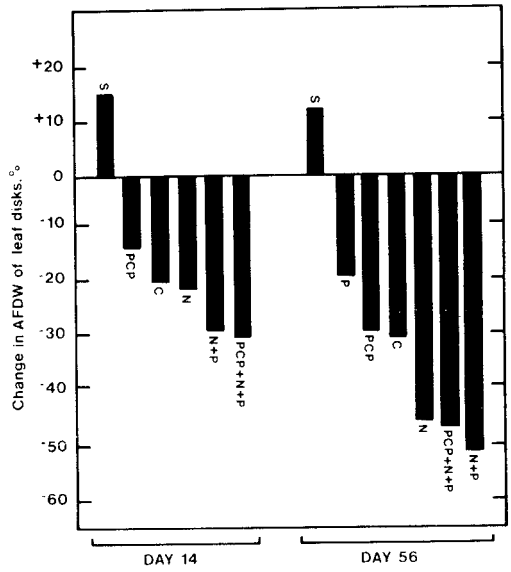

Figure 1. Ash-free dry weight (AFDW) of leaf disks in the sterile control (S), biotic control (C), nitrogen (N), phosphorus (P), nitrogen + phosphorus (N+P), pentachlorophenol (PCP), and pentachlorophenol + nitrogen + phosphorus (PCP+N+P) treatments on days 14 and 56.

The addition of N and N+P significantly increased estimates of biomass, heterotrophic activity, and respiration of microbes associated with leaves and leaf leachates. Treatment with N alone significantly increased measurements of ATP-disk, ATP-water, ^{14}C lipid-disk, ^{14}C lipid-water, and respiration on day 14 (Table 1) as well as measurements of ATP-disk, ATP-water, and respiration on day 56 compared to the biotic control (Table 1). Treatment with N+P stimulated the microbial community to an extent even greater than did the treatment receiving N alone. Several day 14 measurements (ATP-disk, ^{14}C lipid-disk, and respiration) and day 56 measurements (ATP-water and ^{14}C lipid-water) were significantly greater in the N+P treatment than in either the biotic control or N treatments (Table 1).

Increased microbial activity in the N and N+P treatments accelerated loss of AFDW from leaf disks. Leaf disks lost 46 and 52% of AFDW in 56 days in the N and N+P treatments, respectively (Figure 1). However, only the N+P treatment was significantly different from the biotic control in loss of AFDW from leaf disks (Table 2). The leaf disks in the N+P treatment were nearly transparent on day 56 due to the extensive processing that had occurred. In fact, our processing estimate (52%) is probably conservative because many of the leaf disks were completely decomposed and therefore unavailable for analyses. The extent of processing may account for the decrease in respiration, ATP content of leaf disks, and ^{14}C lipid synthesis by disk microbes measured on day 56 in the N+P treatment. The labile elements of the leaf disks had been consumed by the microbial community, leaving only the refractory, structural elements of the leaf disk matrix available for colonization and metabolism by microbes.

Table 1. Measurements of ATP, ^{14}C lipid synthesis, and respiration on days 14 and 56.[a]

	Measurement and day									
	ATP-disk (ng/disk)		ATP-H_2O (ng/ml)		^{14}C lipid-disk (cpm/disk/hr)		^{14}C lipid-H_2O (cpm/ml/hr)		Respiration mg CO_2/flask/wk	
Treatment	14	56	14	56	14	56	14	56	14	56
Control	6.4	12.0	0.39	0.49	6100	5300	1006	763	1.98	1.02
	(0.2)	(2.9)	(0.38)	(0.09)	(583)	(783)	(100)	(323)	(0.02)	(0.05)
Sterile	0.2[b]	.4[b]	0	0.26	966[b]	117[b]	160[b]	83[b]	0.79[b]	0.45[b]
	(0)	(.3)	(0)	(0.25)	(700)	(33)	(163)	(10)	(0.02)	(0.03)
N	23.0[b]	25.9[b]	1.61[b]	1.41[b]	8717[b]	5450	1820[b]	907	5.35[b]	1.44[b]
	(3.9)	(7.7)	(0.32)	(0.37)	(1400)	(833)	(687)	(420)	(0.66)	(0.62)
P	-	(17.0)	-	0.54	-	5917	-	760	-	1.04
		(4.1)		(0.22)		(1733)		(113)		(0.01)
N+P	60.4[bc]	25.3[b]	1.88[b]	1.95[bc]	13483[bc]	6100	1460	1630[bc]	9.43[bc]	1.14
	(5.9)	(2.7)	(0.98)	(0.53)	(817)	(550)	(147)	(247)	(0.20)	(0.10)
PCP	8.6	5.3[b]	0.07	0.53	3100[b]	2233[b]	780	957	2.31	0.94
	(1.0)	(0.8)	(0.08)	(0.25)	(583)	(117)	(183)	(287)	(0.10)	(0.10)
PCP+N+P	19.5[bd]	25.5[b]	0.25[d]	1.15[bd]	6950[d]	6517	1593	1680[b]	9.07[b]	0.84
	(2.4)	(2.5)	(0.20)	(0.29)	(1783)	(1600)	(390)	(317)	(0.20)	(0.07)

[a]Numbers are expressed as mean and standard deviation for n=3 determinations. Standard deviations are in parentheses.

[b]Denotes significantly different from control treatment on individual day at $p \leq .05$ level.

[c]Denotes significant difference between N+P and N treatments on individual day at $p \leq .05$ level.

[d]Denotes significant difference between PCP+N+P and N+P treatments on individual day at $p \leq .05$ level.

Table 2. Measurements of ash-free dry weight, nitrogen, and phosphorus on days 14 and 56.[a]

	Measurement and day											
	AFDW (ug/disk)		N (ug/disk)		N (% of disk)		N (ug/ml H_2O)		P (ug/disk)		P (% of disk)	
Treatment	14	56	14	56	14	56	14	56	14	56	14	56
Control	783	681	10.4	13.9	1.01	1.41	0.23	0.27	0.34	0.57	0.033	0.058
	(164)	(36)	(1.6)	(0.8)	(0.09)	(0.08)	(0.08)	(0.13)	(0.08)	(0.13)	(0.008)	(0.014)
Sterile	1142[b]	1111[b]	11.4	12.1	0.96	0.94[b]	0.11	0.12	0.30	0.28[b]	0.025	0.022[b]
	(160)	(194)	(0.3)	(0.9)	(0.04)	(0.09)	(0.06)	(0.14)	(0.05)	(0.06)	(0.003)	(0.005)
N	772	540	14.2[b]	15.1	1.58[b]	2.08[b]	0.39	0.96[b]	0.37	0.38	0.041	0.053
	(57)	(115)	(2.7)	(1.3)	(0.15)	0.21	(0.10)	(0.20)	(0.13)	(0.36)	(0.011)	(0.050)
P	-	796	-	12.9	-	1.39	-	0.37	-	0.67	-	0.072
		(30)		(1.2)		(0.38)		(0.16)		(0.10)		(0.010)
N+P	696	481[b]	18.8[bc]	14.4	1.90[bc]	2.39[bc]	0.33	1.23[b]	0.97[bc]	0.56	0.096[bc]	0.093[bc]
	(5)	(55)	(1.8)	(1.9)	(0.18)	(0.10)	(0.05)	(0.40)	(0.30)	(0.09)	(0.014)	(0.007)
PCP	851	700	12.9	11.7[b]	1.04	1.39	0.16	0.51	0.44	0.34	0.035	0.040
	(96)	(68)	(1.6)	(0.5)	(0.02)	(0.11)	(0.04)	(0.09)	(0.19)	(0.10)	(0.011)	(0.012)
PCP+N+P	689	525	14.9[bd]	15.0	1.76[b]	2.59[b]	0.64[bd]	1.03[b]	0.66	0.69	0.076[bd]	0.120[b]
	(66)	(128)	(0.4)	(0.6)	(0.23)	(0.17)	(0.22)	(0.17)	(0.22)	(0.01)	(0.014)	(0.004)

[a] Numbers are expressed as mean and standard deviation for n=3 determinations. Standard deviations are in parentheses.

[b] Denotes significantly different from control treatment on individual day at $p \leq .05$ level.

[c] Denotes significant difference between N+P and N treatments on individual day at $p \leq .05$ level.

[d] Denotes significant difference between PCP+N+P and N+P treatments on individual day at $p \leq .05$ level.

Statisically significant trends were also noted in the nitrogen and phosphorus dynamics in the N and N+P treatments. Leaf disks in the N treatment contained significantly more nitrogen (expressed on an absolute basis and on a percentage basis) than biotic control disks on day 14 (Table 2). On day 56 the N treatment was significantly higher than the biotic control in nitrogen percentage of leaf disks and nitrogen content of water (Table 2). However, there was no significant increase in phosphorus content of leaf disks in the N treatment on any date (Table 2).

Leaf disks in the N+P treatment contained significantly more nitrogen and phosphorus on day 14 (expressed on both an absolute basis and on a percentage basis) than did leaf disks in either the biotic control or N treatments (Table 2). On day 56, leaf disks in the N+P treatment remained significantly higher in nitrogen and phosphorus percentage compared to leaf disks in the biotic control or N treatments (Table 2). The N+P treatment was also significantly higher than the biotic control in nitrogen content of water on day 56 (Table 2).

These results are similar to those obtained in experimental recirculating streams by Howarth and Fisher (1976) and imply that leaf decomposition in this study was a nitrogen limited process. When nitrogen was added, however, a limitation of phosphorus occurred because concurrent treatment with nitrogen and phosphorus stimulated microbial leaf processing and nutrient immobilization more than did treatment with nitrogen or phosphorus alone.

Research by Elwood et al. (1981) has indicated that nutrient limitation may also be a significant factor in natural streams. Enrichment of Walker Branch (a second-order woodland stream) with 60 and 450 µg/l P significantly increased weight loss, respiration, and nitrogen content of submerged leaves, which implied a limiting role of phosphorus in detrital leaf decomposition. However, in other studies that compared two Oregon streams, Sedell et al. (1975) and Triska et al. (1975) found differing rates of leaf decomposition which Elwood stated "were apparently due to differing NO_3 concentrations in water," i.e., potentially nitrogen limited conditions.

Although these examples sound somewhat contradictory, Elwood (1981) pointed out that there is probably an interdependence of nitrogen and phosphorus in nutrient limitation of detrital decomposition, and that an optimal ratio of inorganic N:P probably exists somewhere between 1.5:1 and 31:1 for streams similar to Walker Branch. Therefore, a stream with an inorganic N:P ratio that lies below this optimal value would effectively be nitrogen limited; a ratio exceeding this range would indicate phosphorus limitation.

The water in our experiment (control treatment) had an inorganic N:P value of approximately 4:1, a situation that would be expected to be nitrogen limited. Our results confirm this hypothesis, since addition of inorganic nitrogen alone (i.e., N treatment) effectively increased estimates of microbial leaf decomposition and nitrogen immobilization, whereas addition of inorganic phosphorus alone (i.e., P treatment) had no effect. The addition of inorganic nitrogen (i.e., N treatment) effectively raised the inorganic N:P ratio of the water to 111:1, a situation which would become P limited. This was confirmed by simultaneous treatment with N+P. The N:P ratio was decreased in this treatment to 24:1, a ratio within Elwood's optimal predictive range; hence microbial leaf decomposition and nutrient immobilization were further stimulated at this N:P ratio. Thus the results of our nutrient experiment supported Elwood's work, and

indicated that decomposition and nutrient content of detrital leaves may be quite dependent on the amount and ratio of nutrients in streams.

Chemical Perturbation of Microbial Leaf Processing and the Mitigating Effect of Nutrients

The aquatic fungi are the principal microbes that decompose leaves; they are also the principal organisms that increase the protein content of leaves and thus invite invertebrate feeding and processing activity (Hynes et al. 1974, Suberkropp and Klug 1976). Others have indicated that aquatic bacteria are the principal processors of dissolved organic matter derived from terrestrial inputs, leaching, and invertebrate activity (Cummins et al. 1972, Lock and Hynes 1976, Suberkropp and Klug 1976). Hence any toxic compound that alters the numbers or types of aquatic microbes in streams could affect organic matter processing, nutrient retention, and invertebrate production.

Data from the sterile control represent an extreme example of chemical perturbation, in which microbial leaf processing and the concomittant increases in the nutritive value of leaves were checked (Tables 1 and 2). The PCP treatment also indicated, to a lesser degree, that chemical pollutants could disrupt certain processes associated with microbial leaf decomposition. The PCP treatment significantly reduced measurements of ^{14}C lipid-disk on days 14 and 56 (Table 1), ATP-disk on day 56 (Table 1), and nitrogen content of leaf disks on day 56 (Table 2) which implies that the chemical treatment significantly reduced the biomass and heterotrophic activity of leaf disk microbes. A previous study in our laboratory provided similar results, and further indicated that PCP could alter the distribution and succession of aquatic microbes associated with detrital leaves (Fairchild et al. 1983). However, PCP did not affect the loss of AFDW from leaf disks in either study; this lack of effect may imply that this parameter is not a sensitive indicator of chemical perturbation of microbial leaf decomposition.

The addition of nitrogen and phosphorus to the PCP treatment mitigated the apparent effects of pentachlorophenol on the microbial community. In contrast to the PCP treatment, several microbial measurements on day 14 (ATP-disk and respiration) (Table 1) and on day 56 (ATP-disk, ATP-water, and ^{14}C lipid-water) (Table 1) were significantly higher in the PCP+N+P treatment than in the control treatment, indicating that this treatment greatly increased biomass and activity of aquatic microbes. Enhanced microbial activity resulted in increased loss of AFDW from leaf disks in the PCP+N+P treatment; the leaf disks lost 47% of AFDW in 56 days (Figure 1). Although fifty percent greater, this decay rate was not significantly different from the biotic control at the 0.05 level (Table 2).

Nitrogen and phosphorus content of leaf disks also increased in the PCP+N+P treatment, reflecting greater development of microbial protein on the leaf disks. On day 14 leaf disks in the PCP+N+P treatment contained significantly more nitrogen (expressed on a percentage basis and on an absolute basis) and phosphorus (expressed on a percentage basis) than did leaf disks in the biotic control (Table 2). On day 56 the leaf disks in the PCP+N+P treatment still contained significantly higher levels of nitrogen and phosphorus (expressed on a percentage basis) compared to the biotic control (Table 2). Also, the nitrogen content of water was significantly

greater in the PCP+N+P treatment than in the biotic control on both days 14 and 56 (Table 2).

The mitigating effect of nutrients is even more evident when one compares the PCP+N+P treatment with the N+P treatment. Measurements of ATP-disk, ATP-water, and ^{14}C lipid-disk were significantly lower in the PCP+N+P treatment compared to the N+P treatment on day 14 (Table 1); measurements of nitrogen per leaf disk and phosphorus percentage of leaf disks were also significantly lower in the PCP+N+P treatment on day 14 (Table 2). Furthermore, the nitrogen concentration in water was significantly greater in the PCP+N+P treatment than in the N+P treatment on day 14 (Table 2). However on day 56 these two treatments differed significantly in only one parameter: ATP-water (Table 1). Thus by the end of the study there was little difference between measurements in the PCP+N+P and N+P treatments.

The results of this study indicated that inorganic nutrient enrichment increased the resistance or recovery of the microbial community to stress by pentachlorophenol. Similar mitigating effects of nutrients have also been observed in phytoplankton and fungal communities exposed to arsenic (Da Costa 1972, Sanders 1979, Planas and Lamarche 1983).

Neuhold and Ruggerio (1975) contend that post-perturbation recovery (i.e. resilience) is maximal in communities composed of R-selected species such as bacteria, fungi, or phytoplankton. Such communities recover from chemical stress in a relatively short time due to their rapid growth and turnover rates which can facilitate production of species resistant to perturbation (Neuhold and Ruggerio 1975, Pimentel and Edwards 1982) or species which can degrade the chemical stressor in question. This strategy is dependent on high rates of energy and material utilization, processes which are often limited by availability of nutrients.

Thus, it is apparent that inorganic nutrient concentration may be an important factor in determining the vulnerability of an aquatic ecosystem to contaminant stress. Toxic chemicals such as pentachlorophenol could prove most hazardous to microbial leaf processing in pristine, low-nutrient streams draining undisturbed watersheds. On the other hand, toxic elements that enter streams are often of agricultural or domestic origin and frequently enter in concurrence with elevated nutrient levels. These results indicate that pentachlorophenol and perhaps other contaminants may prove less damaging to microbial leaf processing in these nutrient-enriched situations.

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