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Influence of Three Contrasting Detrital Carbon Sources on Planktonic Bacterial Metabolism in a Mesotrophic Lake

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ABSTRACT

Lakes receive organic carbon from a diversity of sources which vary in their contribution to planktonic microbial food webs. We conducted a mesocosm study to test the effects of three different detrital carbon sources (algae, aquatic macrophytes, terrestrial leaves) on several measures of microbial metabolism in a small meso-eutrophic lake (DOC ≈ 5 mg/L). Small DOC additions ($\Delta C < 1$ mg/L) affected bacterial numbers, growth, and pathways of carbon acquisition. Macrophyte and leaf detritus significantly increased TDP and color, but bacterial densities initially (+12 h) were unaffected. After 168 h, densities in systems amended with terrestrial detritus were 60% less than in controls, while production rates in mesocosms with macrophyte detritus were 4-fold greater. Detritus treatments resulted in greater per-cell production rates either through stable cell numbers and greater growth rates (macrophyte-C) or lower densities with stable production rates (terrestrial-C). After only 12 h, rates of leucine aminopeptidase (LAPase) activity were 2.5× greater in macrophyte-C systems than in controls, but LAPase and β-*N*-acetylglucosamindase activities in systems amended with terrestrial-C were only 50% of rates in controls. After 168 h, β-xylosidase rates were significantly greater in communities with terrestrial and phytoplankton detritus. Microbial utilization of >20% of 102 carbon sources tested were affected by at least one detritus addition. Macrophyte-C had positive (6% of substrates) and negative (14%) effects on substrate use; terrestrial detritus had mainly positive effects. An ordination based on carbon-use profiles (+12 h) revealed a cluster of macrophyte-amended communities with greater use of psicose, lactulose, and succinamic acid; controls and algal-detritus systems were more effective in metabolizing two common sugars and cellobiose. After 168 h, communities receiving terrestrial detritus were most tightly clustered, exhibiting greater use of raffinose, pyroglutamic acid, and sebacic acid. Results suggest that pelagic bacterial communities respond to changes in organic carbon source rapidly and by different routes, including shifts in per-cell production rates and variations in degradation of a variety of compounds comprising the DOC pool.

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Introduction

Bacteria are responsible for assimilating most of the dissolved organic carbon (DOC) in aquatic ecosystems, yet the fluxes of DOC through bacteria include a wide variety of compounds derived from unknown sources and composition [4]. Many studies have documented significant seasonal, daily, and diurnal changes in bacterial densities, secondary production, and enzyme activity within microbial communities from a variety of ecosystems [23, 29, 38, 39], despite stable total DOC concentrations [25, 43, 44, 51]. Changes in DOC composition rather than concentration may drive bacterial production and other metabolic processes [23, 45]. These conclusions have been drawn mainly from studies which employ chemical analyses of specific constituents of DOC [43, 48], or are based on the utilization of DOC by bacteria in grazer-free systems [44]. Few studies have directly tested the effects of different sources of organic carbon on community-level bacterial metabolism.

Quantifying the importance of different sources of organic carbon for pelagic microorganisms *in situ* may be complicated by the fact that many small lakes have both high rates of phytoplankton production (a presumed labile DOC source) and substantial supplies of terrestrial sources of carbon (a presumed source of recalcitrant DOC) from the surrounding watershed. It has been argued that in small lakes, dissolved organic matter (DOM) originates primarily from aquatic plants in the littoral zone, as well as from allochthonous (terrestrial) sources [51]. Although littoral macrophyte and allochthonous sources are more recalcitrant than DOM produced by phytoplankton, they may still dominate the carbon metabolism in small lakes. Large pools of allochthonous DOM are thought to contribute to ecosystem stability, while smaller pools of labile forms are produced and used at more rapid rates [21, 48, 51].

The fate and importance of terrestrial sources of DOC have been studied extensively in streams and rivers [26, 28], but have received less attention in lentic ecosystems, despite their probable importance in many small lakes. Forest trees typically provide DOM which is higher in lignin, humic, and phenolic compounds than is detritus from aquatic plants, with less structural carbon. Detritus from aquatic vascular plants supplies dissolved organic carbon capable of supporting high bacterial secondary production in the Hudson River [17], perhaps compensating for a nearly 90% decline in phytoplankton production since the invasion of zebra mussels [9]. The ability of vascular plant matter to support bacterioplankton growth varies with the degradability of the detritus, which can be predicted by differences in cellulose and lignin content, as well as detrital C:N ratios [31].

Studies suggest that humic compounds may satisfy most of the carbon needs of bacterioplankton in humic lakes, although their high C:P and C:N ratios may lead to P or N limitation during growth [20, 21]. Other studies suggest that humics and polyphenols may inhibit certain bacterial enzymes [33, 52]. Whole-ecosystem studies in humic Lake Pääjärvi (Finland) found slight increases in bacterial production in response to moderate additions of humic matter, but no effects on bacterial phosphatase activity or respiration rates [3]. The short-term (hours) and long-term (days) effects of an autumn leaf fall on the metabolism of pelagic microorganisms in uncolored lakes is not well established, but it is likely to be of considerable importance. It has been suggested that a high degree of catabolic flexibility in natural bacterial communities may permit their utilization of a variety of these allochthonous sources of DOC [6, 33]. Experiments are needed to test whether non-phytoplankton DOC may also support bacterial metabolism in uncolored lakes, and whether these sources of carbon may elicit changes in the metabolic and enzymatic activities of pelagic microorganisms.

Phytoplankton, submersed aquatic macrophytes, and terrestrial leaves are each potentially large sources of DOC for bacterioplankton in smaller lakes. The aim of this study was to examine community-level effects of these three contrasting carbon sources on microbial metabolism, measured as thymidine incorporation, enzyme activity and carbon use profiles. An important goal was to examine the effects of these three detrital supplies without making large changes in the total DOC pool, so that responses may be assumed to be the result of carbon source.

Methods

Site Description and Facilities

Studies were conducted during July 1996 at the experimental lakes facility (ELF) at the Louis Calder Center (41°07′40″ N; 73°43′55″ W), the biological field station of Fordham University (Armonk, NY). ELF mesocosms are 5400 L fiberglass tanks (1.9 m high × 2.1 m dia) situated ≈100 m from Calder Lake in a clearing at the edge of a forest, and are lakewater fed by pump. At the time of the study mesocosms were open to normal sunlight an average of 12 h daily. Nylon screens (≈8 mm mesh) prevented leaves and woody debris from entering the mesocosms during experiments. Calder Lake is a small, uncolored, mesoeutrophic, dimictic lake (4 ha, $\overline{Z}$ = 2.8 m, Z_{max} = 6.7 m), with a submersed macrophyte community (mainly *Vallisneria americana, Elodea canadensis, Potamogeton amplifolius,*

Najas flexilis) covering more than 50% of the lake bottom. Summertime epilimnetic chlorophyll *a* concentrations average 3–5 μg/L and epilimnetic DOC concentrations average ≈5 mg/L [25, 49, 50]. Because of its small size, the lake receives a proportionately large subsidy of leaves from forest trees (mainly *Quercus rubra, Q. prinus, Acer rubrum, Fagus grandifolia, and Cornus florida*) in the autumn. Bacterial production rates are typically limited by the supply of inorganic P and/or N [25, 46, 50].

Experimental Design

Experiments were run in 16 ELF tanks that had been filled with whole lakewater (all planktonic organisms), pumped from Calder Lake at a pelagic station at ≈1 m depth during the night. Because only planktonic responses were under study, mesocosms contained no benthic community. The experimental design compared bacterial responses to different sources of detrital carbon, at equivalent levels of added DOC. The target was to increase DOC concentration by 1–2 mg/L (within the range of seasonal variation). Laboratory studies of DOC release by each carbon source determined the total amount of each detritus source required to amend DOC concentration by an equal amount. The four detritus treatments were (a) algae, (b) submersed macrophytes, (c) terrestrial leaves, and (d) no addition (control); each was replicated four times. Algal detritus was prepared by enriching Calder Lake phytoplankton (over 2 weeks) in four ELF tanks (5400 L) with surplus levels of N and P, then pumping the entire volume of each tank through a 20 μm mesh plankton net. Algal concentrates (including zooplankton, heterotrophic protists) were heat-killed in an autoclave (16 PSI, 125° C, 1 h) and allowed to cool; one "algal addition" (12 mg POC/L) came from an entire ELF tank (possible changes in algal POC due to autoclaving were regarded as preferable to adding live cells). Macrophyte detritus was prepared by collecting late-summer submersed plants in proportions found in the lake (50% *Vallisneria*, 40% *Elodea*, 10% *Potamogeton*). The plants were dried at 105°C and ground in a Wiley mill (≈0.5 mm mesh). Each of four mesocosms received 18.5 mg (dry mass) aquatic macrophyte detritus per L to achieve the target DOC addition. Terrestrial detritus was prepared by collecting freshly fallen forest leaves in proportions approximating forest composition adjacent to the lake (65% *Quercus*, 25% *Acer*, 10% *Fagus* + *Cornus*), drying, and grinding material as described above; four tanks each received 15 mg terrestrial detritus/L. Treatments were added at night in random order while tanks were being filled.

Sampling

Mesocosms were sampled during two periods to measure metabolic responses of bacteria in early (12 h) and late (168 h) phases of detritus decomposition. Lakewater was collected using a peristaltic pump at ≈0.5 m depth and stored cold (≈4°C) until it could be processed in the laboratory (<4 h). Unfiltered water was collected for bacterial samples (numbers, production, metabolic assays) and light absorption characteristics (UV-B absorption, color). Water chemistry samples were collected in an identical manner but filtered in-line using a 47 mm Nalgene filter unit fitted with a Poretics (GF-75 ≈ 0.7 μm) glass fiber filter to remove particulate matter (prior comparisons with 0.45 μm pore size filters yielded similar values). Water chemistry samples were collected in acid-washed vials, acidified to pH <2.0 with H_2SO_4, and stored at 4°C until analysis. Temperature and O_2 levels were monitored (0.5 m depth only) using a YSI (model 57) meter.

Physical and Chemical Analyses

Water chemistry samples were analyzed for soluble-reactive (SRP) and total dissolved (TDP) phosphorus using antimony–ascorbate–molybdate [1, 7]; TDP was measured as for SRP following in-line acid-persulfate digestion with high-energy UV light. Dissolved organic carbon (DOC) was measured (after DIC removal via acid sparging) following digestion with acid persulfate and high-energy UV; the CO_2 produced was dialyzed and reacted with buffered phenolphthalein and measured at 550 nm [8, 19]. These procedures were run on a TrAAcs 800 automated analyzer (Bran+Luebbe Inc., Buffalo Grove, IL). The light absorbing properties of water in each treatment were considered from two perspectives: UV-B absorbance (at 310 nm) and color (in Pt units). For both, water was filtered using a 0.2 μm pore size filter immediately prior to measurements (≥8 h of collection) using a Shimadzu UV-160 spectrophotometer, with matched quartz cuvets. Color was estimated from absorbance measurements converted to color values (Pt units), based on the equations of Cuthbert and del Giorgio [14].

Biological Analyses

Bacteria samples (1 mL sample + 14 mL distilled H_2O) were preserved with glutaraldehyde (2% final concentration), stained with DAPI, and filtered onto Irgalan black 25 mm polycarbonate filters (0.2 μm pore size; Poretics Corp.) for enumeration [34]; slides were stored frozen (−15°C) until counted. Counts were made using an epifluorescence microscope (Nikon Labophot) with a UV-2A filter set (excitation 330 nm; barrier 420 nm) at 1000× magnification. At least 10 grids and 300 cells were counted per sample. Bacterial production rates were measured (168 h only) using the rate of [^{3}H]thymidine ([^{3}H]TdR) incorporation into DNA [16]. Ten-mL subsamples were incubated with 40 μCi [^{3}H]TdR (final conc 50 nM) for 1 h (linear incorporation period) in a 25°C water bath; samples and zero-time controls were stopped using 5% formalin. After incubation, cells were filtered onto 0.2 μm pore size polycarbonate filters, rinsed with cold 5% trichloroacetic acid, and frozen prior to DNA extraction [16]. Radioactivity (^{3}H) levels were measured using a Beckman LS1801 liquid scintillation counter.

Mixed bacterioplankton assemblages were screened for community-level metabolic properties using the Biolog microplate system [5, 6, 18, 37, 24, 41], which was originally designed as a means of identifying bacterial isolates based on substrate utilization profiles (Biolog, Inc., Hayward, CA). Unfiltered lakewater (135 μL) from each mesocosm was added to each of 96 wells in the GN microplate, which contained 95 different organic carbon sources plus a negative control (distilled H_2O). A second MT microplate was em-

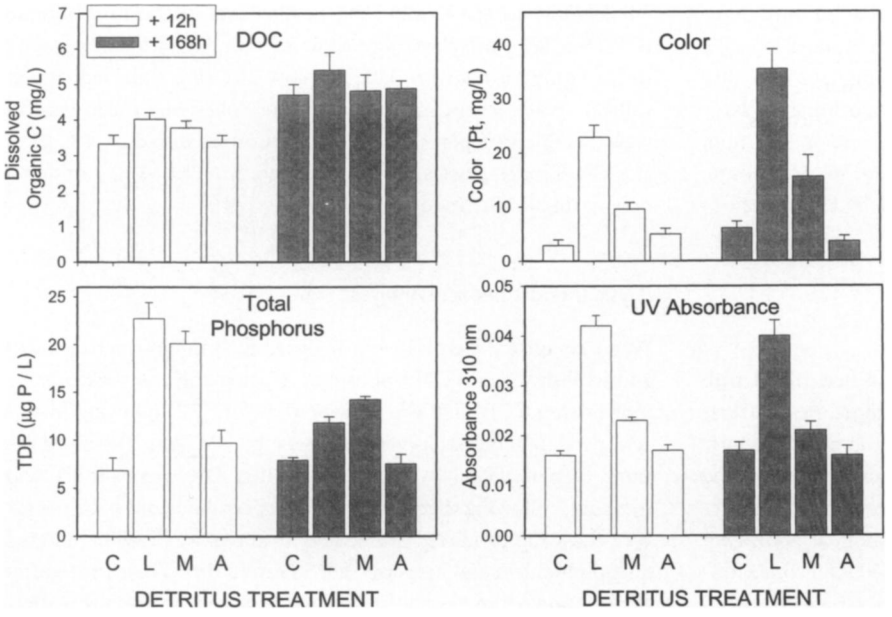

Fig. 1. Dissolved organic carbon (DOC), color (Pt units), total dissolved phosphorus (TDP), and UV-B absorbance in Calder Lake mesocosms amended with different detrital carbon sources, measured 12 h and 168 h after addition (C = controls, L = terrestrial leaves, M = macrophyte, A = algae; bars are means of 4 replicates + 1 SE).

ployed, to which seven additional substrates (based on their likelihood of contributing to the total DOC pool in lakes) were added: cellulose (plant cell walls), chitin (invertebrate exoskeleton), benzoic acid, ferulic acid, cinnamic acid (lignin derivatives), and capric acid and stearic acid (bacterial cell walls). This produced a suite of 102 different substrates tested as sole carbon sources. Plates were incubated at ≈24°C for 2–3 days until maximum color was achieved; optical density (OD) is a function of respiration rate measured in each well. OD was measured (less control well blanks) using a Bio Kinetics microplate reader (model EL-340, Biotech Instruments, Inc.) at 590 nm. A second set of assays measured enzyme activity rates (nmol substrate consumed per unit volume h^{-1}) of eight specific enzymes: α-1,4-glucosidase (αGase); β-1,4-glucosidase (βGase); β-xylosidase (βXase); β-*N*-acetylglucosaminidase (NAGase); alkaline phosphatase (APase); esterase (Ace); leucine aminopeptidase (LAPase); and endopeptidase (ENDase). Assays were run in black 96-well microplates, using 250 µL of unfiltered lakewater from two replicate mesocosms (per carbon treatment) for each enzyme tested. Incubations ran for 3.8 h and were measured (0, 1, 1.8. 3.8 h) using a Perkin-Elmer fluorometer. Because of a malfunction in a freezer during storage, only two replicates could be analyzed for enzyme activities. Further details of methods are described elsewhere [40].

Data Analysis

The effects of detritus source (algae, macrophyte, terrestrial, control) on selected physicochemical and physiological variables were considered using analysis of variance (ANOVA). Data were checked for assumptions of normality and homogeneity of sample variances prior to analysis; no transformations were required. Tukey's Honestly Significant Difference (HSD) compared (*post hoc*) individual treatment effects, if the general ANOVA led to a rejection of the null hypothesis [42]. For both procedures, effects were judged to be significant if the probability (P) of a type I error (α) was <0.05. Data from community-level (Biolog) assays of 102 substrates were compiled as a 16 case (4 treatment × 4 replicate) by 102 variable matrix for an ordination of communities based on their physiological profiles [12, 18, 37]. A principal components analysis (PCA) was performed using covariance as a distance measure [27]. Component loadings were correlated against Biolog OD values for each of the 102 carbon sources to identify which substrates exerted the greatest influence in differentiating community types. Data were complied and analyzed using SYSTAT v. 7.0 for Windows [53].

Results

Chemical and Physical Changes

Mesocosms maintained similar temperature conditions (daytime mean 24.5°C) among treatments, but dissolved oxygen levels were significantly depressed within 12 h in systems receiving terrestrial and macrophyte detritus (≈60% saturation; $P < 0.001$). Detrital treatments had small effects on dissolved organic carbon (DOC) levels (Fig. 1). After 12 h, average DOC concentrations in systems supplemented with detrital-C had increased by only about 0.2 to 0.7 mg/L. No significant differences in DOC concentrations were measured among the four detrital treatments at either time period (Table 1). However, the quality of DOM changed substantially; detritus-amended mesocosms had substantial increases in total dissolved phosphorus (TDP; Fig. 1). Because of the different effects of treatments on TDP and DOC, the

Table 1. Effects of detrital carbon source on nutrient concentrations, light absorbance characteristics, and bacterial density and production rates in planktonic communities from Calder Lake, measured 12 and 168 h after carbon additions to mesocosms[a]

Variable	Time (h)	Treatment effect		Treatment differences
		F	*P*	least → most
DOC	12	2.46	0.113	C ≈ A ≈ M ≈ L
	168	0.82	0.968	C ≈ A ≈ L ≈ M
TDP	12	13.03	<0.001	C ≈ A < M ≈ L
	168	0.56	0.653	C ≈ A ≈ L ≈ M
Color	12	82.32	<0.001	C ≈ A < M < L
	168	27.89	<0.001	A ≈ C < M < L
UVB absorption	12	75.47	<0.001	C ≈ A < M < L
	168	22.54	<0.001	C ≈ A < M < L
Bacterial density	12	0.53	0.671	L ≈ C ≈ A ≈ M
	168	4.39	0.026	L < C ≈ A ≈ M
Bacterial production	12	ND		ND
	168	81.50	<0.001	C ≈ L < A < M

[a] Treatment effects tested by ANOVA; *F* = calculated *F*-ratio, *P* = probability; ND = no data. Comparison of individual treatment differences are listed in order of magnitude (C = control, A = algal detritus, M = macrophyte detritus, L = terrestrial leaf detritus; comparisons based on Tukey's HSD; "≈" signifies no significant difference, $P > 0.050$)

C:P ratios of dissolved organic matter changed substantially, averaging ≈1,400 in control systems (extreme P-deficiency) and ≈400 to 500 in terrestrial and aquatic-macrophyte amended systems (moderate P-deficiency). Greatest TDP levels (lower C:P ratios) were measured in systems receiving terrestrial leaves (+15.9 µg P/L, 2.3-fold increase; $P < 0.001$) and aquatic macrophytes (+13.3 µg P/L, 2.0-fold increase; $P < 0.001$). Enhanced TDP levels continued even after 168 h, although increases were somewhat less (≈50% greater + terrestrial detritus; ≈80% greater + macrophyte detritus; differences NS, Table 1). No measurable effect of algal detritus was observed for either nutrient.

While DOC concentrations were similar among treatments after 168 h, there were small but significant changes in color in mesocosms supplied with terrestrial and macrophyte detritus (Fig. 1). The water appeared slightly brown in terrestrial-C treated mesocosms throughout the experiment; data suggest this effect was more pronounced later in the study (+168 h). Greater color values corresponded with greater UV-B (310 nm) absorbance (effects highly significant, $P < 0.001$), with greatest values in mesocosms with terrestrial detritus, followed by modest increases in systems amended with detritus from aquatic macrophytes (Table 1).

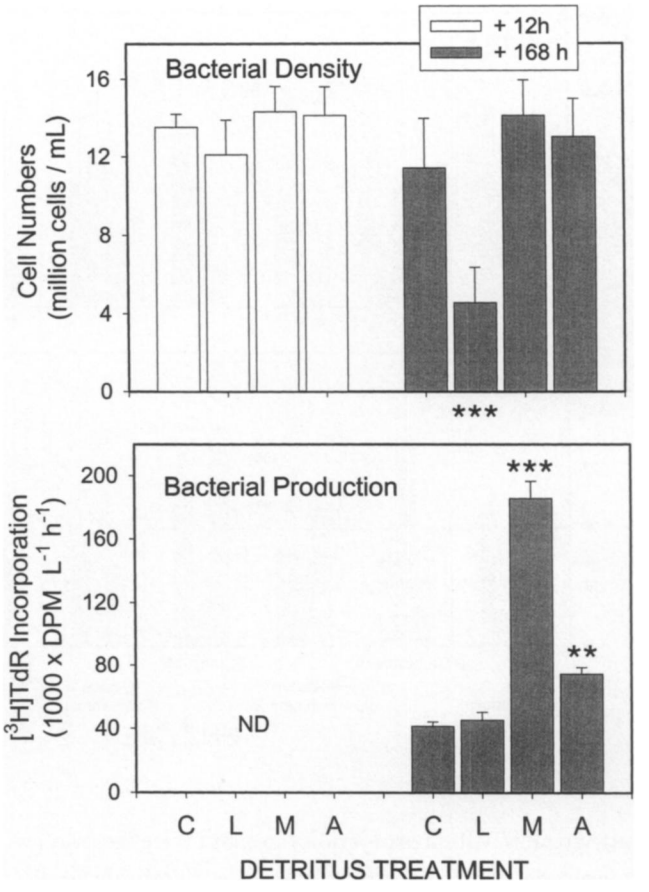

Fig. 2. Densities and production rates of bacterioplankton in Calder Lake mesocosms amended with different detrital carbon sources, measured 12 h and 168 h after addition (see Fig. 1 for detritus types; mean + 1 SE; ND = no data available; asterisks above bars = positive effects, below bars = negative effects; ** = significantly different from controls, $P < 0.01$; *** = $P < 0.001$).

Bacterial Density and Production Rates

Early in the study (+12 h), numbers of bacterial cells were not significantly affected by detritus treatment (Fig. 2). Densities were high, but all values were within ±10% of the overall average of ≈13.5×10^6 cell/mL. After 168 h, numbers were largely unchanged and unaffected by treatments, except in mesocosms amended with terrestrial detritus, which had declined by >60% (significantly different from all other treatments, $P < 0.001$). Patterns of bacterial production rates (TdR incorporation, 168 h only) were also significantly affected, but did not parallel results for cell numbers (Fig. 2). Bacterial production rates were nearly 4.5 times greater in communities that received macrophyte-C than in controls ($P < 0.001$), despite similar cell numbers (density difference NS). Mesocosms receiving algal detritus also had signifi-

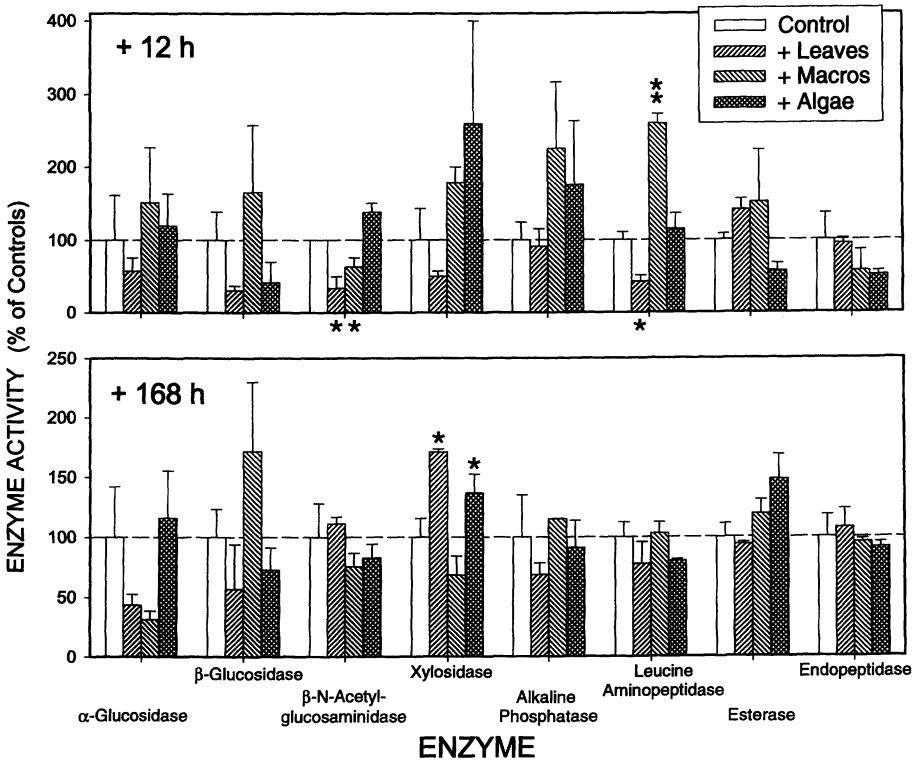

Fig. 3. Relative enzyme activities of eight ectoenzymes, as influenced by different detrital carbon sources. Rates were measured in communities collected 12 h and 168 h after detrital additions (means of 2 replicates + 1 SE; asterisks above bars = rates significantly greater than controls; asterisks below bars = rates significantly less; * = $P < 0.05$; ** = $P < 0.01$).

cantly greater TdR incorporation rates, but the effect was less pronounced (1.8 × controls; differences significant at $P < 0.001$).

Enzyme Activity

For most enzymes, activities increased on average ≈15-fold between 12 and 168 h after detrital additions (Fig. 3). For example, early in the study APase activity (across all treatments) averaged ≈0.0158 nmol mL^{-1} h^{-1}, but increased nearly tenfold to ≈0.160 nmol mL^{-1} h^{-1} after 168 h. Average rates of α- and β-Gase activity initially were also low (e.g., macrophyte mesocosms at 12 h: 0.62 and 2.1 μmol mL^{-1} h^{-1}, respectively) but increased by 5- to 10-fold over this time (after 168 h: 6.23 and 10.8 μmol mL^{-1} h^{-1}, respectively). Only ENDase activity was found to be greater (≈2.9 × greater at 12 h) early in the study. Significant treatment effects were few, owing to low replication levels ($n = 2$; see Methods), but the magnitude of measured differences among detritus treatments were large for several enzymes (Fig. 3, upper panel). In the early (12 h) phase of decomposition, average rates of α- and β-Gase activities were 50% greater in mesocosms supplied with macrophyte detritus than in controls, while APase (NS) and LAPase (P = 0.002) activities more than doubled. In contrast, rates of nearly all enzymes decreased to ≤50% of controls in systems supplied with terrestrial leaf detritus. Rates of LAPase activity declined to ≈40% of controls ($P = 0.037$). In general, 12 h enzyme activities in systems amended with algal-detritus supplemented systems were similar to controls.

Later in the decomposition process, enzyme rates and detrital treatment effects changed (Fig. 3, lower panel). There were fewer stimulatory effects of macrophyte detritus, with only one of eight enzymes more than 20% greater than controls (β-Gase activity 1.7-fold greater, diff. NS). And unlike the early phase, measured activities of three enzymes (α-Gase, NAGase, βXase) were reduced by at least 25% versus control communities. The broad negative effect of terrestrial leaf detritus on ectoenzyme activity observed early in the study had largely reversed by 168 h, with a few stimulatory responses (e.g., βXase activity was 1.7-fold greater, $P = 0.034$). At this time, βXase activity was also significantly greater (1.5-fold, $P = 0.038$) in algal-detritus amended than in control communities.

Patterns of Substrate Use

Assays of sole-carbon use revealed strong and rapid changes (both enhanced and suppressed) in bacterial metabolism in response to detritus treatments (Figs. 4, 5). Within 12 h, the use of 21 of 102 substrates tested were significantly affected (Table 2). Macrophyte detritus mainly stimulated activity

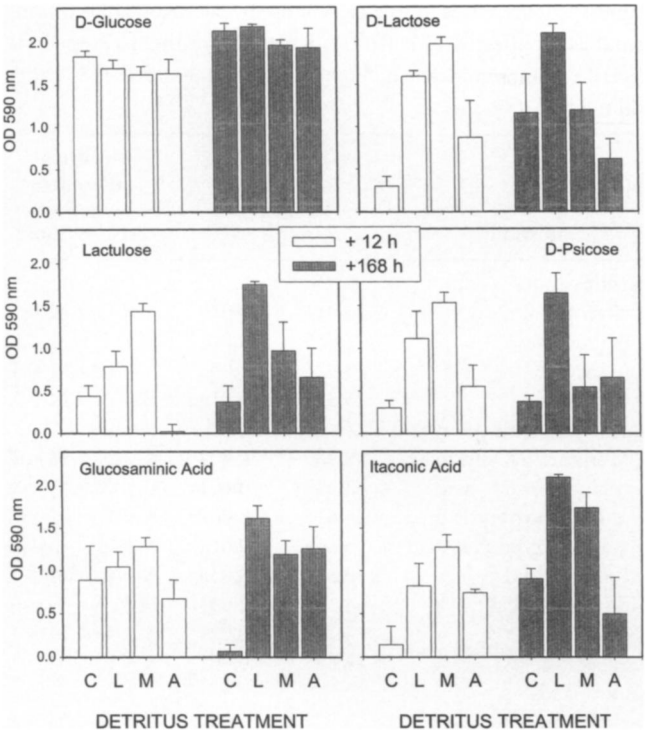

Fig. 4. Metabolism of six different carbohydrates and carboxylic acids as sole carbon sources by mixed bacterioplankton communities in Calder Lake mesocosms 12 h and 168 h after detritus additions (see Fig. 1 for detritus types; means of 4 replicates + 1 SE). Optical density is a function of respiration activity (see Methods) following utilization of substrates provided (all plotted to same scale). Treatments are the initial experimental detrital additions to each mesocosm.

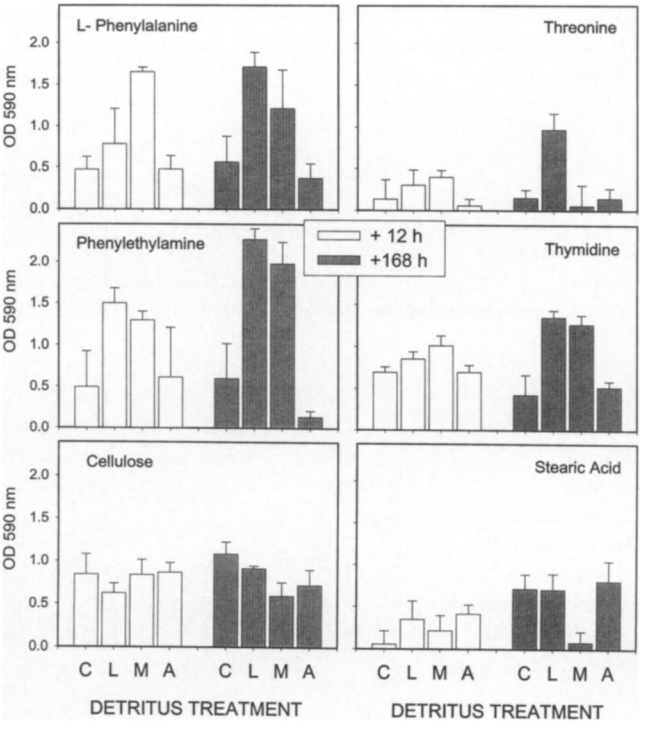

Fig. 5. Metabolism of selected amino acids, amines, and cell wall components as sole carbon sources for mixed bacterioplankton communities collected from Calder Lake mesocosms 12 h and 168 h after detritus additions (see Fig. 4 for details).

(overall average: 1.8-fold increase), and enhanced C metabolism by as much as 26-fold (α-ketovaleric acid), and more than 3-fold greater for 10 different substrates. Utilization of a few substrates, such as maltose and mannose, was reduced (≈30% less) versus control communities. In this early phase, use of several substrates by communities amended with terrestrial leaf detritus were 20 to 30% lower than controls (e.g., succinamic acid, D-alanine, urocanic acid). As a group, carbohydrate use was most affected, although simple sugars (e.g., glucose) was easily metabolized by bacteria and largely unaffected by treatments (Fig. 4). Despite evidence of P-limitation in the available DOM (based on high initial C:P ratios), utilization of glucose 6-phosphate was also unaffected by detritus treatments. In contrast, metabolism of complex carbohydrates was strongly affected by detrital additions. For example, lactose utilization was 6.6× greater in macrophyte-amended communities, 5.3× greater following terrestrial detritus additions, and nearly 3-fold greater with algal detritus ($P < 0.001$; Table 2). In contrast, metabolism of lactulose was 90% less in systems amended by algal detritus than controls, but was stimulated by macrophyte (3.4×) and terrestrial leaf (1.8×) treatments. Few positive effects were observed with addition of algal detritus, although utilization of some carboxylic acids was greater (e.g., itaconic acid use was 5-fold greater than controls at 12 h). In this earlier phase, no treatment effects were seen for any of the cell wall components tested in the MT plates, and these were metabolized much less than nearly all other substrates (Fig. 5).

Community substrate-use patterns shifted after 168 h, with detrital treatments significantly changing utilization of 25 different carbon sources (Figs. 4, 5). But unlike the early phase, substrate utilization was most often greatest in mesocosms which received terrestrial leaf detritus (19 of 25 significant effects; Table 3). The largest differences were observed with the degradation of carboxylic acids; values ranged from 2.3-fold (itaconic acid) to 24-fold (glucosaminic acid) greater in leaf-amended communities than in controls. At this time, effects of terrestrial leaf detritus on amino acid degradation were most consistent. Utilization of nearly 40% of amino acids tested were significantly greater than in controls (mean effect size = 2.8× greater). Results for

Table 2. Differences in sole-carbon use by bacterioplankton communities, as affected by different detrital-C treatments, measured 12 h after addition[a]

Substrate	Treatment effect		Treatment differences
	F	*P*	least → most
Carbohydrates			
Dextrin	4.773	0.021	M < L ≈ A ≈ C
N-Acetyl-galactosamine	4.876	0.019	M < A ≈ L ≈ C
D-Lactose	10.383	0.001	C ≈ A < L ≈ M
Lactulose	23.135	<0.001	A ≈ C < L < M
Maltose	4.488	0.025	M < L ≈ A ≈ C
Mannose	3.714	0.042	M < L ≈ A ≈ C
Psicose	6.581	0.007	C ≈ A < L ≈ M
Xylitol	5.059	0.017	C ≈ A < L ≈ M
Carboxylic acids			
Itaconic acid	6.349	0.008	C < A ≈ L ≈ M
α-Ketovaleric acid	14.399	<0.001	A ≈ C ≈ L < M
Bromosuccinic acid	5.400	0.014	C ≈ L ≈ A < M
Succinamic acid	10.913	0.001	A ≈ C ≈ L < M
Amino acids			
D-Alanine	5.785	0.011	A ≈ L ≈ C < M
L-Alanine	4.401	0.026	A ≈ L < C ≈ M
L-Asparagine	3.957	0.036	M < A ≈ L ≈ C
Glycyl-L-glutamic acid	5.605	0.012	A ≈ C < L ≈ M
L-Phenylalanine	5.261	0.015	C ≈ A < L ≈ M
L-Serine	4.145	0.031	M ≈ A < C ≈ L
γ-Aminobutyric acid	4.087	0.033	A ≈ C < M ≈ L
Other			
Urocanic acid	3.904	0.037	L ≈ A ≈ C < M
2-Aminoethanol	4.582	0.023	L ≈ C ≈ A < M

[a] Substrates listed are those whose metabolism (based on OD) was found to be significantly different ($P > 0.050$) among treatments (treatment effects tested by ANOVA; F = calculated F-ratio, P = probability). Comparison of individual treatment differences are listed in order of magnitude (C = control, A = algal detritus, M = macrophyte detritus, L = terrestrial leaf detritus; comparisons based on Tukey's HSD; "≈" signifies no significant difference, $P > 0.050$)

Table 3. Differences in sole-carbon use by bacterioplankton communities, as affected by different detrital-C treatments (tested via ANOVA, only significant differences listed), measured 168 h after addition[a]

Substrate	Treatment effect		Treatment differences
	F	*P*	least → most
Carbohydrates			
Glycogen	5.911	0.010	A < C ≈ M ≈ L
D-Fructose	5.663	0.012	M ≈ L ≈ A < C
D-Lactose	3.925	0.036	A ≈ C ≈ M < L
Lactulose	5.246	0.015	C ≈ A < M ≈ L
Carboxylic acids			
Monomethyl succinate	9.085	0.002	A ≈ C < M ≈ L
D-glucosaminic acid	15.409	<0.001	C < M ≈ A ≈ L
α-Hydroxybutyric acid	26.615	<0.001	A ≈ C ≈ L < M
γ-Hydroxybutyric acid	5.668	0.012	A ≈ C < L ≈ M
Itaconic acid	3.865	0.038	A ≈ C ≈ M < L
D,L-Lactic acid	4.154	0.031	M < A ≈ L ≈ C
Quinic acid	4.743	0.021	A ≈ C < M ≈ L
Sebacic acid	18.682	<0.001	A ≈ C < M ≈ L
Amino acids			
L-Glutamic acid	3.922	0.037	C ≈ A < M ≈ L
L-Leucine	4.886	0.019	A ≈ C ≈ M < L
L-Phenylalanine	4.058	0.033	A ≈ C < M ≈ L
L-Proline	3.690	0.042	A ≈ C ≈ M < L
L-Pyroglutamic acid	3.884	0.038	A ≈ C < M ≈ L
L-Threonine	6.036	0.010	M ≈ A ≈ C < L
D,L-Carnitine	7.390	0.005	A ≈ C < M ≈ L
γ-Aminobutyric acid	9.021	0.002	C ≈ A < M ≈ L
Amines			
Inosine	3.748	0.041	C ≈ A ≈ M < L
Thymidine	9.020	0.002	C ≈ A < M ≈ L
Phenylethylamine	19.549	<0.001	A ≈ C < M ≈ L
Other			
Ferulic acid	7.073	0.005	M < A ≈ L ≈ C
Stearic acid	4.819	0.020	M < L ≈ C ≈ A

[a] Comparisons of individual treatment differences (tested via Tukey's HSD) are listed in order of magnitude (see Table 2 for details)

carbohydrates also differed from the earlier time period. For example, lactose metabolism was nearly 2-fold greater with leaf detritus than in both controls and macrophyte-treated mesocosms (earlier rates greatest with macrophyte detritus). A comparison of 12 h vs 168 h utilization patterns overall found 2- to 5-fold increases in use of most substrates, although use of some C sources was substantially reduced (e.g., oxidation of glucosoaminic acid in controls declined to <10% of 12 h levels). Results for 168 h also indicate that the metabolism of some bacterial wall components were significantly, and negatively, affected by detrital additions (Table 3); decomposition of stearic and ferulic acids was more than 80% lower in communities supplied with macrophyte detritus (Fig. 5). Additions of algal detritus again resulted in the fewest effects on carbon use patterns (use of two substrates negatively affected).

Ordination of Bacterioplankton Communities Based on Carbon Use

Physiological similarities among bacterial assemblages were recognized using principal components analysis (PCA: Fig. 6, upper panel). PCA was efficient in describing these relationships; the first two component axes explained >82% of the total variance in the data. Early in the study (12 h), there was no clear distinction among control and algal detritus communities based on carbon use patterns. The most tightly

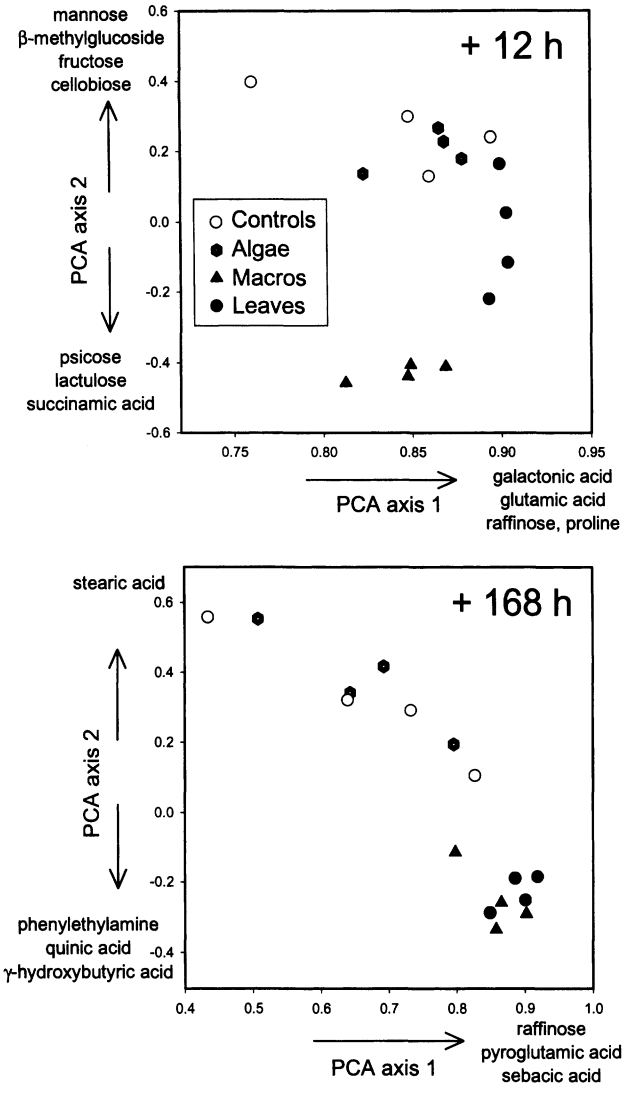

Fig. 6. Principal components analysis of bacterioplankton communities based on their use of 102 sole-carbon sources, 12 h and 168 h after receiving different detrital treatments. Carbon sources listed along axes were substrates most strongly (+ and −) correlated with component loadings (all $P < 0.010$, except stearic acid, $p = 0.029$).

clustered group at 12 h consisted of the four communities which received macrophyte detritus. These assemblages were also the least similar to the control assemblages or communities receiving one of the other two sources. Correlations between individual substrate use and PCA loadings along the first two axes identified which carbon sources were most important in separating the communities. Although carbon use patterns for bacterioplankton receiving terrestrial leaf detritus were not as closely grouped as those receiving macrophyte detritus, their position along PCA axis 1 (74% of total variation in data) indicates these microorganisms used several amino acids and raffinose to a greater degree than communities receiving other treatments. Microbial communities amended with macrophyte detritus were distinguished by greater use of lactulose, psicose, and succinamic acid. Control and algal-detritus treated systems were more effective in metabolizing two common sugars (fructose, mannose; PCA axis 2) and cellobiose (sugar unit of cellulose).

After 168 h, similarities among bacterial communities had changed (Fig. 6, lower panel); the first two PCA axes explained ≈70.5% of the total variation in these data. The terrestrial leaf treatment group was much more distinct from controls and algal-detritus communities after the longer decomposition period, and had the greatest overall similarity among replicate mesocosms. Microbial communities receiving aquatic macrophyte detritus remained physiologically distinct from those in algal and control mesocosms and now plotted in the same general region as those amended with terrestrial leaf detritus. Axis 1 (60.6% of total variation) was primarily a gradient of lower to higher use of raffinose, pyroglutamic acid, and sebacic acid, with macrophyte and leaf-amended communities plotting positively. These same communities were poor degraders of stearic acid, a component of bacterial cell walls, but strong degraders of two carboxylic acids and one amino acid (phenylethylamine). At no time were more recalcitrant carbon sources (e.g., cellulose, chitin) degraded at appreciably different levels among the communities compared, despite receiving different detritus treatments.

Discussion

General Effects

Our goal was to quantify microbial responses to changes in the quality of organic carbon available in the total DOC pool. Additions of three detritus sources had small effects on DOC concentration, but profound effects on other chemical conditions in lakewater and microbial metabolism. Within 12 h after the addition of terrestrial leaf and macrophyte detritus, we measured a nearly 3-fold increase in TDP (organic P ≈ 67% of TDP), increasing the %P of DOM by more than 2-fold. As production rates of lake bacteria are often limited by P availability [13, 25, 46], the TDP:DOC ratio may be a good measure of DOM quality. Stoichiometric patterns are useful indexes for ecosystem processes [15]. Limited data for Calder Lake mesocosms (168 h, $n = 16$) suggest a significant positive correlation ($r = 0.580$, $P = 0.018$) between TDP:DOC ratios and bacterial production

rates. Further studies are needed to determine if a general empirical relationship exists (e.g., Redfield ratio [35]: 106C: 16N:1P) between bacterial metabolic rates and TDP:DOC.

During decomposition of detritus additions there were there were no increases in bacterial densities, but a substantial decline (>70% less than controls) was observed were in response to terrestrial POC after 168 h (Fig. 2). Growth of aquatic bacteria can be suppressed by humic substances and other terrestrially derived organic compounds [47]. Predation may also account for these declines, but densities of bacterial grazers in leaf-amended mesocosms were indistinguishable (heterotrophic, mixotrophic flagellates) or less (rotifers) than in controls (Wehr, unpublished). In the present study bacterial production rates in terrestrial-detritus amended systems (168 h) were indistinguishable from those in controls on a per-volume basis, but were actually 2.5× greater than rates in controls on a per-cell basis. At the same time, bacterial production rates in mesocosms receiving macrophyte detritus were 4.5-fold greater than controls, but with similar cell densities. Results suggest that bacterial communities may respond to changes in the source of organic carbon by different routes. When terrestrial DOM is supplied, community density may decline, but shift to species capable of assimilating recalcitrant DOM with rapid per-cell production rates. A supply of labile DOM from aquatic macrophytes may result in stable densities with high total productivity, but lower per-cell activity.

Links between Production and Community Composition

As microbial densities at 12 h were indistinguishable among treatments and DOC levels changed by <1 mg/L, differences in enzyme activities and substrate use were either due to shifts in metabolic pathways within the community or changes in community composition. This issue has been considered in soil microbial communities [37], with some studies showing evidence of successional change [36], and others suggesting that substrate utilization patterns may simply measure the response of particular bacteria capable of responding to specific C sources [6].

Species shifts are a plausible explanation in Calder Lake for two reasons. First, microplate assay systems are used to characterize strains of bacteria based on their use of a suite of carbon sources [2, 5]. Second, we have screened more than 120 bacterial isolates from Calder and other lakes for carbon-use patterns and have found that physiological profiles match closely with genetic identities (>50 sequences), based on 16s rDNA sequences (Petersen and Wehr, unpublished data). However, recent studies caution that C utilization assays of natural community samples may incur methodological biases due to selection for specific (rapidly growing) bacteria in microplate wells, and may not test the most ecologically relevant substrates [24, 41]. Nonetheless, our results differ from prior studies which compare individual environmental samples. Here we collected large volumes (5400 L) of replicated plankton communities, amended with one of four detritus treatments. The primary selection force acting on Calder Lake assemblages was POM added to the mesocosms. We interpret the metabolic shifts as a response to detrital POM and microbial-driven changes in the quality of organic matter.

Inferred changes in the Calder Lake microbial community composition corresponded with continued degradation of POM by 168 h, as evidenced by greater separation of control and algal-treated assemblages from those receiving macrophyte and terrestrial leaf detritus (Fig. 6). For example, terrestrial detritus at first inhibited microbial activity (measured in three independent assays), but a community later developed which included species able to degrade compounds present in terrestrial leaves (greater per-cell TdR incorporation, enzyme activity, substrate use). Further, the lag in response to terrestrial DOM suggests a possible feedback mechanism: any pre-existing labile DOC in Calder lakewater may first need to be depleted before changes in carbon acquisition pathways are induced.

Enzyme Activities and Sole-Carbon Use by Bacterioplankton

Significant and often large differences in enzyme activities and carbon use patterns among communities receiving different sources of DOM indicate that bacterial metabolism is highly variable and responsive to ecological conditions. For example, rates of endopeptidase (ENDase) activity were greatest early in the study, while the rates for other enzymes (especially α-1,4-glucosidase and alkaline phosphatase) were greater after several days. APase rates initially were low, but increased over time coupled with declining levels of TDP, suggesting that P limitation may have been important later in this study. Lowest APase rates were measured in systems supplied with terrestrial leaves, which received the greatest P release (Fig. 1). Rates of general carbohydrases, such as α- and β-glucosidase, and NAGase activities were initially stimulated by macrophyte detritus, while terrestrial DOM suppressed α- and β-Gase activity. Rates of β-Gase activity have been shown to respond positively to supplies of phytoplankton-derived DOC and glucose [10, 11], suggesting

enzymes may be "switched on" by easily degradable macromolecular C sources, such as decomposing aquatic macrophytes.

It is possible algal detritus stimulated the utilization of very few substrates because our experiment was conducted in July, when the planktonic community may receive the bulk of its DOC from algal sources. For mesocosms receiving macrophyte or terrestrial leaf detritus, there were large and rapid differences in the use of more than 20 substrates. For example, lactose utilization was 2.9-, 5.3-, and 6.6-fold greater in mesocosms supplied with algal, terrestrial leaf, and macrophyte detritus than in controls. In contrast, another carbohydrate (dextrin) was metabolized less effectively in detritus-amended communities, at rates 65% to 75% of those measured in controls. As DOC concentration and bacterial numbers did not change substantially during this period, we suggest these data are evidence that the C acquisition properties of the community had shifted.

Carbon use profiles have been applied to a few aquatic microbial communities [12, 18], but none have yet tested experimentally the influence of different ecologically relevant organic carbon sources (detritus) on these patterns. In the early phase of decomposition when it may be assumed that more labile carbon sources could be decomposed, we found that sugars and carboxylic acids were the most common carbon sources metabolized to a greater extent when supplemented by macrophyte detritus (Figs. 4, 5; Table 2). Our data support earlier suggestions that bacterioplankton are able to shift ectoenzyme synthesis rapidly (within 12 h) to match the supply of utilizable macromolecular organic matter [11]. Rates of extracellular α- and β-1,4-glucosidase activity in the coastal Mediterranean varied more than an order of magnitude diurnally and is thought to be a response to short-term changes in photosynthetic activity and extracellular release of DOC [22].

Partially degraded leaf litter may provide enough DOC and inorganic nutrients for microbial use in autumn and winter, when algal or macrophyte sources may be reduced. Indeed, bacterial biomass and heterotrophic activity in eutrophic Plußsee (Germany) and humic Lake Mekkojärvi (Finland) are similar despite 4-fold greater phytoplankton production in the former and 3-fold greater DOC in the latter [33]. A large pool of mostly recalcitrant DOM in humic lakes can represent the majority of the total DOC for microbial processes. Some studies suggest that a predominance of recalcitrant allochthonous matter would result in a decline in bacterial growth and metabolic activity [47]. However, the present study suggests that several days after its addition, terrestrial detritus can support rapid per-cell bacterial production rates (Fig. 2) and enhanced enzyme activities (Figs. 4, 5, Table 3).

Effects of terrestrial DOM can be complex and changeable. Humic matter additions to limnocorrals in a lake in northern Manitoba inhibited primary production, but relieved P-limitation [20]. Tranvik has pointed out the paradoxical effects of allochthonous DOM on bacterioplankton, in that many lakes with greater inputs of recalcitrant DOM also have high bacterial biomass [47]. Humic substances contain a variety of α- and β-linked carbohydrates, and inhibitory substances such as polyphenolics [33]. Recalcitrant DOM may also be degraded through photolysis by natural levels of UV irradiance alone [32, 52] and, in combination with microbial activity, can result in more complete decomposition of both low and higher molecular weight humic compounds [30]. In Calder Lake, different effects of allochthonous DOM were revealed at different time scales. The initial inhibition by humic materials (reduced enzyme activity and substrate use) was later replaced by enhancement through the catabolic flexibility of the community.

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