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Evidence for the use of non-detrital dissolved organic matter by microheterotrophs on plant detritus in a woodland stream

J. CLARK MILLER Institute of Ecology, University of Georgia, Athens, Georgia, U.S.A.

SUMMARY. 1. Recent studies provide evidence for the use of exudates from living plants by epilithic microheterotrophs in streams. This study investigated the possible use of such non-detrital sources of dissolved organic matter (DOM) by stream microheterotrophs colonizing leaf litter. Biomass of bacteria and of fungi accumulating *in situ* on autumn-shed leaves in flow-through troughs from which light was excluded was compared to that accumulating on leaves in troughs open to natural illumination.

2. In experiments repeated at different times of year and in different stream sections, greater biomass of microheterotrophs consistently accumulated on the leaf detritus in troughs open to natural illumination. Differences in water temperature or in grazing of leaf surfaces by macroinvertebrates could not account for these consistent differences. Further, greater microheterotroph biomass accumulated on light- and dark-incubated leaves in a stream section relatively open to sunlight, compared to corresponding leaves in a section heavily shaded by canopy and understorey vegetation.

3. These and other results suggest that, to some yet undetermined extent, detritus-associated microheterotrophs use non-detrital DOM. This conclusion is consistent with *a priori* predictions based on consideration of microbial energetics involved in the use of detrital versus non-detrital DOM.

4. Studies of trophic pathways in streams and other aquatic habitats have failed to assess some potentially important sources of non-detrital DOM. The ability of available techniques to assess the relative roles of detrital and non-detrital sources of DOM is evaluated, and alternative approaches to this problem are suggested.

Introduction

Woodland streams are believed to exemplify an ecological feature common to many other aquatic habitats: trophic dependence of most animal biomass on dead plant matter (Fisher & Likens, 1973; Hynes, 1975; Mann, 1975). In some cases aquatic consumers are thought to assimilate significant amounts of plant detritus directly (e.g. Findlay & Tenore, 1982). However, a preponderance of evidence against this direct trophic link (e.g. Hargrave, 1970;

Correspondence: J. Clark Miller, Rt 1 Box 127K, Baker, OR 97814, U.S.A.

Rossi & Fano, 1979) has usually led to the conclusion that heterotrophic bacteria and fungi which colonize plant detritus act as trophic intermediates between detritus and detritus ingesters. On this view, these microheterotrophs utilize detrital DOM leached from detritus and/or released by their own enzymatic degradation of the refractory detritus to which they attach (Cummins, 1974; Kirchman, 1983; Alongi & Hanson, 1985). The microbially incorporated detrital organic matter is then utilized, it is thought, as relatively assimilable microbial biomass. These trophic pathways form the basis for the presumed importance of plant detritus in forested streams (Cummins, 1974), lakes (Wetzel, 1975), estuaries (Morrison & White, 1980), salt marshes (Mann, 1982) and other aquatic habitats (Fenchel, 1970; Tenore & Rice, 1980).

Some evidence, however, suggests the possible importance of alternative, non-detrital sources of DOM for detritus-associated microheterotrophs. Increasing numbers of studies suggest that in streams (Geesey *et al.*, 1978; Haack & McFeters, 1982; Rounick & Winterbourn, 1983; Winterbourn, Rounick & Hildrew, 1986) and in other aquatic habitats (Bell, 1983; Søndegaard, 1983; Pakulski, 1986) planktonic and epilithic microheterotrophs readily utilize the labile DOM released by aquatic microalgae and macrophytes. There are no apparent reasons to suspect that detritus-associated microheterotrophs would be less likely to utilize these non-detrital sources of DOM. In fact, consideration of microbial energetics and seasonal dynamics of DOM available to detritus-associated microheterotrophs in streams leads to the hypothesis that non-detrital DOM may be preferentially utilized. In contrast to leaf leachate, DOM released from algae consists mostly of labile, low-molecular-weight compounds (e.g. Kaplan & Bott, 1985). Release of DOM by stream algae (Bott & Ritter, 1981), and apparently also by macrophytes (Wetzel, 1975), occurs over the annual cycle as a normal

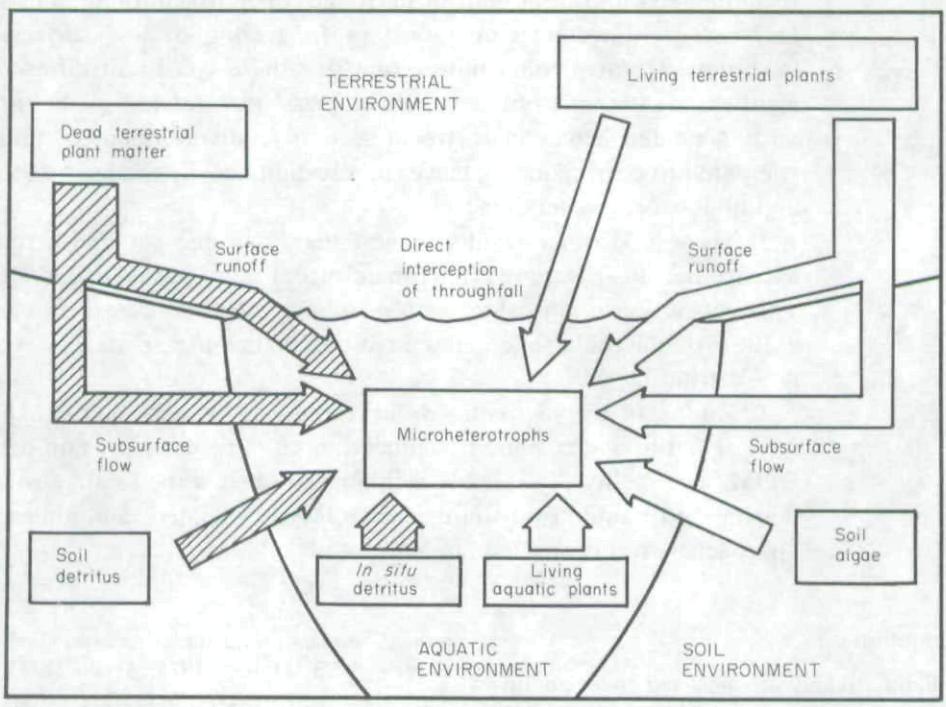

FIG. 1. Sources of detrital (hatched arrows) and non-detrital (open arrows) DOM potentially utilized by unattached microheterotrophs and by microheterotrophs on detritus and other surfaces in aquatic habitats. Detrital DOM may be released by microbial enzymatic attack and/or leached from dead particulate plant matter in the aquatic, soil or terrestrial environments. Non-detrital DOM may be leached and/or exuded from living plants in the aquatic, soil or terrestrial environments.

part of their physiology. In contrast, the labile component of terrestrial leaf litter is leached rapidly (Nykvist, 1963; Kaushik & Hynes, 1971) resulting in a temporary pulse of labile detrital DOM during autumn leaf fall (McDowell & Fisher, 1976). Thus during most of the year use of detrital DOM by detritus-associated microheterotrophs would require metabolism of refractory molecules taken from the medium and/or extracellular breakdown of refractory detritus. From an energetic standpoint, preferential use of the relatively labile non-detrital DOM pool would be predicted. Accordingly, experimental studies (Bengtsson, 1982, 1983) have shown that: (1) simple carbon compounds are effectively utilized by fungi on dead leaves for growth when available in dissolved form, (2) fungal extracellular protease activity is repressed by the addition of such compounds, and (3) at least some fungi on dead leaves use dissolved carbon compounds from the medium when available rather than attacking leaf structural components.

This study was undertaken to assess indirectly the possible importance of non-detrital DOM for growth of microheterotrophs on dead leaves in a woodland stream. As used here, 'detrital' refers to DOM originating from dead particulate plant matter; 'non-detrital' refers to DOM leached or exuded from living plants. Detrital and non-detrital sources of DOM potentially utilized by aquatic microheterotrophs are represented in Fig. 1.

Study site

The study was conducted within the Coweeta Hydrologic Laboratory (U.S. Forest Service, Macon County, North Carolina, U.S.A.). The stream in which experiments were undertaken is a typical small (*c.* 1 m channel width), steep gradient, southern Appalachian Mountain stream shaded by a mixed hardwood canopy and with a stream bed dominated by rock outcrop and large substrate particles. A dense rhododendron (*Rhododendron maximum* L.) understorey provides additional shading along much of the stream length. All but one experiment were conducted in a reach heavily shaded by deciduous canopy and rhododendron understorey (site A). One experiment was undertaken in a downstream reach lacking understorey and receiving greater insolation (site B).

Methods

At the beginning of each experiment, a plastic mesh bag (*c.* 5 mm mesh size) containing ten red maple (*Acer rubrum* L.) leaves was tacked to the bottom of each of two plywood troughs (*c.* 20×60×10 cm deep) which previously had been placed near each other in the stream. A black plastic cover and interior baffles at both ends prevented natural illumination from entering the experimental (darkened) trough, while allowing current flow. The control trough was identical to the darkened trough, but without any covering.

Two types of red maple leaves were used: (1) leaves which had been collected shortly before or after autumn abcission and stored dark and dry, and (2) leaves allowed to undergo microbial colonization outdoors on the ground for several months before use. In both cases light- and dark-incubated leaves were allowed to accumulate microbial biomass in the stream for 4 weeks. Temperature of water flowing over leaves was recorded prior to removal of leaf bags. Bags were removed carefully so that macroinvertebrates would not be lost and could be later quantified from the same leaves on which microheterotrophs were quantified. A metal sieve (1 mm mesh) was placed under trough outflows to catch any animals dislodged during bag removal. Any sediments left in troughs under where bags had been were also washed into the sieve. Leaf bags and associated sediments were placed in separate containers with stream water for transport to the laboratory.

Transport time to the laboratory was less than 10 min. Contents of each leaf bag were placed in separate pans, and discs (12 mm diameter) were cut randomly from light- and dark-incubated leaf material. For subsequent direct observations of fungal hyphae, twenty discs each from light- and dark-incubated materials were stained immediately with alcoholic lactophenol cotton blue (Shipton & Brown, 1962). For quantification of bacteria, ten discs each from light- and dark-incubated materials were placed individually in glass test

tubes with 19 ml prefiltered (0.22 μm) water and sonicated for 10 min in an ultrasound bath. Discs were then removed and 1 ml formalin was added to samples containing cells and other material agitated from leaf disc surfaces. All remaining materials from each leaf bag were placed in separate containers with associated sediments and stream water, fixed with formalin (*c.* 10% of total volume), and stored until macroinvertebrates were removed.

Fungal hyphae were counted along random transects (on both sides of each disc) using a bottom-illuminated binocular microscope. Bacterial cells were counted in filtered subsamples stained with acridine orange (AO) using epifluorescence microscopy. The procedure followed for staining and enumeration of bacteria is that described by Porter & Feig (1980). Macroinvertebrates from leaf bags and associated sediments which may have fed on microbes on leaf surfaces were counted and their lengths were measured. Their biomass was estimated using available weight–length relationships (Miller, 1985).

The experimental hypothesis that less heterotrophic microbial biomass would accumulate on darkened leaves rests on the premise that exclusion of natural illumination and consequent reduction of available non-detrital DOM would negatively affect survival and/or growth of colonizing microheterotrophs. However, numerical abundance of fungal hyphae along transects or of bacterial cells cannot be assumed to provide a direct measure of microbial biomass, because individual hyphae and bacteria may vary in size. To compare microbial biomass on light- and dark-incubated leaves, bacterial rods and cocci were counted separately, and counts of fungal hyphae were made at two magnifications (240× and 500×). Statistical tests for differences between light- and dark-incubated material were then made separately for each of these microbial size ranges/morphological types.

All statistical comparisons were made using the Wilcoxon rank sums test, a distribution-free procedure. In all cases the null hypothesis of no difference in microbial abundance between light- and dark-incubated leaf material was tested against the alternative hypothesis of greater microbial abundance on light-incubated leaves.

Results

In experiments undertaken in autumn and winter, bacterial cells sonicated from leaf surfaces were more abundant in samples from light-incubated leaves (Table 1). No statistical difference (alpha=0.05) was detected in abundance of cocci from light- and dark-incubated leaves in the experiment begun 13 December. All other differences, including abundance of bacterial rods in the 13 December experiment, were statistically significant. Thus total bacterial biomass was consistently greater on light-incubated leaves.

Fungal hyphae, particularly the largest and most distinct, appeared to be somewhat aggregated near leaf veins, as reported also by Paul *et al.* (1977). At 240×, only larger hyphae, which typically appeared to anastomose, were visible clearly. At 500×, counts also included hyphae which were smaller in diameter, less distinct, typically shorter, and often not anastomosing. It was not determined whether these smaller hyphae were actually fungal, or may have been filamentous bacteria (Actinomycetales). For convenience, all stained filaments which were observed are referred to as 'fungal hyphae'. For both of the above size ranges, greater density of hyphae accumulated on leaves incubated with natural illumination available in experiments undertaken in spring at two different sites, and these differences were highly significant (Table 2). In addition to comparing hyphal abundance on light- and dark-incubated leaf material at each of the two sites (Table 2), the following comparisons were made: (1) the hyphal abundance on light-incubated leaves at the heavily shaded site was compared to abundance on light-incubated leaves at the more open site, and (2) hyphal abundance on dark-incubated leaves at the heavily shaded site was compared to abundance on dark-incubated leaves at the more open site. More hyphae accumulated on light-incubated leaf surfaces at the site receiving greater insolation ($P<0.001$, $P=0.018$ for counts at 240× and 500×, respectively). Greater density of larger hyphae accumulated on dark-incubated leaves at the more open site ($P<0.001$ for counts made at 240×). At 500×, no difference in density of hyphae on dark-incubated leaves was detected between sites ($P=0.436$). Thus for all statistical comparisons

TABLE 1. Relative abundance of bacterial cells (median number per ten microscope fields) accumulating *in situ* over 4 weeks on control leaf litter in troughs open to natural illumination (Light) and on leaf litter in troughs from which natural illumination was excluded (Dark). Leaves used in the 3 October experiment were stored dark and dry until use. Leaves used in other experiments were pre-colonized (see Methods). Values in parentheses are lower and upper limits of 95% confidence intervals for medians.

Beginning of experiment	Water temperature at mid-day*			Relative number of bacterial cells					
				Rods			Cocci		
	Light	Dark	*n*	Light	Dark	*P*	Light	Dark	*P*
3 October 1982	14	14	6	1136 (1012, 1192)	739 (684, 949)	0.008	1597 (1196, 2265)	1131 (232, 1443)	0.021
7 November 1982	9	9	7	1712 (1551, 1870)	1318 (1216, 1436)	<0.001	610 (368, 838)	358 (238, 577)	0.049
13 December 1982	8	9	10	1202 (1085, 1347)	933 (868, 1088)	0.003	423 (292, 475)	287 (232, 473)	0.083

*Temperature of water flowing over leaves measured on day of collection.

TABLE 2. Relative abundance of fungal hyphae (median number per four 12 mm transects) accumulating *in situ* over 4 weeks (16 April to 14 May 1983) on control leaf litter in troughs open to natural illumination (Light) and on leaf litter in troughs from which natural illumination was excluded (Dark). Leaves were stored dark and dry until use. Values in parentheses are lower and upper limits of 95% confidence intervals for medians.

Site	Water temperature at mid-day*		Number of fungal hyphae							
			Counted at 500×				Counted at 240×			
	Light	Dark	*n*	Light	Dark	*P*	*n*	Light	Dark	*P*
A (heavily shaded)	12	13	10	398 (333, 433)	244 (205, 272)	<0.001	20	130 (116, 153)	68 (63, 91)	<0.001
B (more open)	12	13	10	446 (409, 534)	260 (222, 322)	<0.001	20	196.5 (185, 226)	128 (114, 159)	<0.001

*Temperature of water flowing over leaves measured on day of collection.

TABLE 3. Total numbers and biomass (mg fresh weight) of macroinvertebrates collected from the same leaves on which microheterotrophs were quantified. Taxa included were only those whose feeding modes may have included removal of microbes from leaf surfaces.

Beginning of experiment	Number		Biomass	
	Light	Dark	Light	Dark
7 November 1982	14	10	2.8	2.1
13 December 1982	57	40	1.3	22.0
16 April 1983				
Site A	199	100	49.8	10.7
Site B	267	73	36.1	32.8

of fungal abundance, greater total fungal biomass accumulated on light-incubated leaf litter compared to dark-incubated, and on litter incubated in the stream section more open to natural illumination.

Macroinvertebrates associated with the leaves on which microheterotrophs were quantified were dominated numerically by chironomid larvae. Most samples also included a number of late-instar mayfly (Ephemeroptera) nymphs which, because of their greater size relative to chironomids, contributed most to total biomass. Total numbers and biomass in light-incubated leaf bags compared to dark-incubated bags was variable (Table 3). For each of the four experiments in which abundance of these macroinvertebrates was quantified, a different result was obtained: (1) similar total numbers and biomass in light and dark-incubated leaf bags (7 November experiment), (2) similar total numbers but greater biomass in the dark-incubated leaf bag (13 December experiment), (3) greater numbers and biomass in the light-incubated leaf bag (16 April experiment, site A), and (4) greater numbers in the light-incubated bag but similar biomass in light- and dark-incubated bags (16 April experiment, site B).

Discussion

Factors possibly influencing abundance of microheterotrophs on light- and dark-incubated leaf litter

While photosynthetic activity was prevented in the darkened troughs and unaffected in the controls, it is assumed that there was no systematic difference between control and darkened troughs in availability of detrital DOM to microheterotrophs. Mesh bags themselves presumably had some effect on light reaching leaves. However, compared to natural stream processes (e.g. leaves turning over, deposition of silt and coarse debris on leaves) this effect was not likely significant. Conceivably, it is possible that in a given experiment comparing microbial abundance on leaves in a control and a darkened trough, greater microheterotroph biomass on light-incubated leaves could have been due to chance rather than any effect related to photosynthetic processes. For example, recently-fallen leaf litter might by chance have drifted into the control trough, providing a miniature pulse of detrital DOM to light-incubated leaves, resulting in greater microbial biomass. However, it is clear that this type of event cannot account for the consistent finding of greater biomass of microheterotrophs on light-incubated leaves in five separate experiments (Tables 1 and 2).

Nor can water temperature or removal of bacteria or fungi from leaf surfaces by macroinvertebrates account for the consistently greater biomass of microheterotrophs on light-incubated leaves. Temperature of water flowing over leaves in troughs at mid-day was always either equal or up to 1°C higher in the darkened trough (possibly due to heat absorption by the black cover). If differences in abundance of microheterotrophs between light and dark troughs was a result of grazing of leaf surfaces by macroinvertebrates, abundance of grazers would be expected to have been greater on leaves on which microheterotroph abundance was found to be reduced (dark-incubated leaves). Inconsistent with this explanation, numbers and biomass of macroinvertebrates which may have grazed leaf surfaces were greater on light-incubated leaves or similar on light- and dark-incubated in all but one case (Table 3).

Results of comparisons between leaves incubated in control and darkened troughs suggest that non-detrital DOM released by microalgae within control troughs was utilized by bacteria and fungi colonizing dead leaves. Plywood surfaces in control troughs immediately upstream from leaf bags provided approximately 0.1 m^2 surface area for algal colonization, growth and release of DOM. In darkened troughs, photosynthetic activity on this corresponding surface area and by microalgae col-

onizing the leaves themselves (Iverson, 1973; Suberkropp & Klug, 1974; Paul *et al.*, 1977) would have been prevented.

Further evidence suggests that leaf surface microheterotrophs utilized additional non-detrital DOM from sources outside the immediate environment of the troughs. The fact that fungal biomass was greater in the darkened trough located in the more open stream section compared to the darkened trough in the heavily shaded section suggests that fungal growth on the dead leaves was influenced by the relative concentration on non-detrital DOM entering darkened troughs in the streamwater. Supporting this conclusion, heterotrophic microbial activity in darkened, flow-through tubes was somewhat greater in a stream section with high algal primary productivity compared to activity in darkened tubes in a stream section with much less primary production (Peterson *et al.*, 1985).

The comparison made between fungal biomass accumulating on leaves in the control trough located in the more open stream section to that on leaves in the control trough in the heavily shaded section constituted a 'natural' experiment which complemented the 'controlled' light versus dark comparisons (Miller, 1986). Results of this natural experiment (greater fungal biomass in the stream section more open to natural illumination) reinforce results of the light/dark comparisons by suggesting that the observed effect on biomass of microheterotrophs was not simply due to some unrecognized difference between control and darkened troughs. In turn, results of comparisons between control and darkened troughs paired in the same stream location suggest that the effect observed in the natural experiment was not simply due to some unrecognized difference between the more open and more shaded sites.

The complementary lines of evidence discussed above support the hypothesis that, to some yet undetermined extent, detritus-associated microheterotrophs in aquatic habitats use non-detrital DOM. This conclusion is consistent with predictions based on microbial energetics and with the known trophic coupling of planktonic (e.g. Bell, 1983) and epilithic (Haack & McFeters, 1982) microheterotrophs (which are intimately associated with fine particulate plant detritus) with non-detrital sources of DOM. Evidence available at present is not sufficient for assessment of the relative importance of detrital and non-detrital sources of DOM. It may be significant, however, that the observed reduction of microheterotroph biomass when photosynthetic activity was prevented (Tables 1 and 2) occurred in late autumn, when availability of labile detrital DOM is greatest (McDowell & Fisher, 1976), and also in winter, when photosynthetic production by stream plants is minimal (e.g. Benfield, 1981).

Although this is the first study to address the role of non-detrital DOM in the nutrition of detritus-associated microheterotrophs, evidence from some other studies supports the conclusion reached here. A decrease in fungal abundance due to exclusion of light was reported for twigs colonized in a midwestern U.S.A. stream (Shearer & Von Bodman, 1983). Metabolism (uptake of acetate) of microheterotrophs associated with algal cells in detrital aggregates has been shown to be greater in the euphotic zones of lake and marine environments than in the aphotic (Paerl, 1973, 1974). Marine detrital aggregates containing intact diatoms gave rise to bacterial growth in culture and appeared to support bacteria *in situ*; in contrast, other aggregates characteristically devoid of algal cells were also nearly free of microheterotrophs and did not support bacterial growth in culture (Wiebe & Pomeroy, 1972).

Assessment of the trophic importance of detrital versus non-detrital DOM

Evidence from this study and others noted above conflicts with a large number of studies which have been interpreted as support for the view that woodland streams and other aquatic habitats are detritus-based. It points to the need for experimental approaches which can resolve the question of the relative trophic roles of detrital and non-detrital sources of DOM.

A body of evidence indicates that fungi on plant litter are able to breach plant tissue enzymatically and invade it with hyphae. This is often interpreted as indicating the importance of detritus in their nutrition (e.g. Bärlocher & Kendrick, 1974). However, two alternative hypotheses can also account for this phenomenon. Even if these fungi were relying

on non-detrital DOM rather than the detrital DOM released, penetration of litter would still be advantageous as (1) a means of stable attachment to a surface where algal production and exudation occurs, or (2) a refuge from the activity of invertebrates removing microbes from leaf surfaces.

In a related approach, microheterotrophs are often exposed to a source of detrital DOM under unnatural conditions, and some measure of microbial activity (e.g. uptake, respiration) is obtained (e.g. Ladd *et al.*, 1982). This type of result is typically interpreted as evidence for microheterotroph use of detrital DOM in nature. However, this approach conflicts with basic principles of microbial ecology and physiology. The potential of microheterotrophs to utilize a wide variety of substances through physiological adaptation is well known (e.g. Alexander, 1965). Through enzyme induction, population growth and/or activation of inactive populations, a natural assemblage of microheterotrophs can acclimate trophically to a variety of sources of DOM, whether or not these sources are utilized normally in the natural environment (e.g. Kaplan & Bott, 1985). Thus, in experiments in which the normally available pool of non-detrital and detrital DOM is artificially altered, utilization of experimentally added DOM cannot be taken as evidence for utilization of the experimental DOM source in nature. This is most obviously the case in experiments in which microheterotrophs are isolated in darkened experimental chambers and then, for example, exposed to radiolabelled detrital DOM (e.g. Ladd *et al.*, 1982; Benner, Moran & Hodson, 1985; Findlay, Smith & Meyer, 1986). In these experiments, in which normally available, labile, non-detrital DOM is excluded and only detrital DOM is available, it is not surprising that some of the experimentally supplied detrital DOM would be utilized. This type of result does not indicate that the experimentally supplied detrital DOM is actually utilized in nature. The same type of artificial alteration of the natural DOM pool and resulting acclimation of microheterotrophs to detrital DOM would be expected to occur in darkened stream sections (Kaplan & Bott, 1983; Kuserk, Kaplan & Bott, 1984), in darkened flow-through tubes (Peterson *et al.*, 1985), and in enclosed recirculating chambers isolated from natural sources (Fig. 1) of non-detrital DOM (e.g. Dahm, 1981).

A more recent approach to analysis of aquatic habitat trophic pathways is stable carbon isotope analysis (e.g. Rounick, Winterbourn & Lyon, 1982). Although these studies have allowed some discrimination between aquatic and terrestrial sources of organic matter for stream food webs (e.g. Winterbourn *et al.*, 1986), for a number of reasons this approach is not capable of resolving the relative importance of detrital versus non-detrital DOM. First, no study has yet obtained $\delta^{13}C$ values for all the potentially important sources of detrital and non-detrital DOM (Fig. 1), including DOM released by all aquatic plants and DOM leached from terrestrial plants. Second, in at least some habitats (e.g. Winterbourn *et al.*, 1986), $\delta^{13}C$ values for aquatic primary producers are such that trophic use of their exudates alone or in combination by microheterotrophs would possibly result in $\delta^{13}C$ values in consumer tissue indistinguishable from those which would result if leaf litter DOM was being utilized.

Most importantly, it is impossible from this type of analysis to distinguish between utilization of DOM originating from dead plant litter (detrital) and DOM leached and/or released from the living plants which produce the litter (non-detrital). For example, stable carbon isotope ratios of consumers in streams are typically closer to ratios of tree leaf tissue in stream sections lined by the corresponding tree species (e.g. Rounick *et al.*, 1982; Winterbourn, Cowie & Rounick, 1984; Winterbourn & Rounick, 1985). However, it is not possible from these studies to distinguish between two possible hypotheses to account for these results: (1) that at these sites greater amounts of detrital DOM from leaf litter are available to microheterotrophs and consequently to consumers, or (2) that at these sites greater amounts of DOM leached from tree leaves by rainfall (Fig. 1) enter the food web via microheterotrophs. Dissolved organic matter leached from tree leaves and entering streams directly or indirectly via throughfall (Fig. 1) is one potentially important source of non-detrital DOM which has not yet been assessed. However, in a north-central U.S.A. deciduous forest receiving 80 cm annual precipitation, soluble carbohydrates in throughfall amounted

to 35–59 kg ha^{-1} yr^{-1} (McClaugherty, 1983), much of which is composed of sugars (Parker, 1983). Direct input of such non-detrital DOM to a stream overhung by canopy vegetation would be equivalent to 3.5–5.9 g m^{-2} yr^{-1}, and presumably would be greater in streams in areas with much higher annual rainfall (e.g. Winterbourn *et al.*, 1984).

An alternative to the above general approaches involved observation of bacterial growth on gradient plates in which dissolved organic carbon (DOC) from two different sources (pre-abscission deciduous tree leaf leachate and leachate from dead prairie grass) was used (McArthur, Marzolf & Urban, 1985). Results of this study indicated that bacteria in upper, grassland reaches of the stream (where tree litter was absent) had the genetic/enzymatic potential to use both grass leachate and deciduous leaf leachate. However, only bacteria from lower stream reaches, where microheterotrophs would have been exposed to tree leaf leachate, grew on gradient plate areas where concentration of tree leaf leachate was highest. These results and others (Kaplan & Bott, 1985) suggest that the physiological potential of microheterotrophs to utilize various types of DOC is influenced by the range of DOC sources in their environments. This type of study, however, does not address the question of the relative importance to microheterotrophs of detrital and non-detrital DOC *in situ*.

Possible approaches to resolving the relative trophic importance of detrital versus non-detrital DOM

Because it is clear that present approaches cannot unequivocally resolve the trophic importance of detrital versus non-detrital DOM in aquatic habitats, alternative experimental methods are required. An apparent solution to the problem of physiological acclimation in studies of utilization of DOM by microheterotrophs is to (1) use samples of microheterotrophs taken from their unaltered habitat and thus physiologically acclimated to the natural DOM pool of their environment rather than to a pool which is an artefact of the experimental method, and (2) measure the immediate microheterotroph response to an experimentally enriched source of DOM, since eventual acclimation to and utilization of the supplied DOM is likely to occur regardless of whether it is being utilized in nature. If in nature the microheterotrophs are utilizing the experimentally introduced DOM, stimulation of uptake and oxidation of substrate is immediate (e.g. Vaccaro & Jannasch, 1966; Williams & Gray, 1970). (This immediate response forms the basis of the uptake-kinetic method of measuring microheterotroph activity.) If the experimentally enriched DOM is not being utilized in nature, there should be no immediate response, followed in approximately 2–3 days (Kaplan & Bott, 1985) by a response due to acclimation.

This pattern of no immediate response followed by a delayed response was obtained by Rounick & Winterbourn (1983) when leaf leachate at a level 10 times greater than total DOC concentrations in a forested stream was added to samples of stream-incubated litter. This appears to be the only study to date in which the two necessary requirements noted above were met: (1) lack of prior acclimation to an artificially altered DOM pool in which non-detrital DOM is eliminated or greatly reduced, and (2) measurement of immediate microbial response. The observed response indicates that the detritus-associated microheterotrophs in this forested stream were not utilizing DOM from leaf detritus *in situ* at the time of the year that they were taken from the stream.

Stable carbon isotope ratio analysis might also be capable of resolving the detrital versus non-detrital question if used in novel experimental approaches. Some differences in $\delta^{13}C$ values between terrestrial deciduous trees do occur. For example, Rau & Anderson (1981) obtained a value of $-29‰$ for alder leachate, compared to $-25.3‰$ for beech leachate (Rounick *et al.*, 1982). Consider two artificial stream channels fed by natural stream water and colonized by stream flora and microflora, but each side supplied with equal amounts of two different species of leaf litter differing in $\delta^{13}C$ value. Each channel would also be provided with early instars of a species of stream macroinvertebrate which feeds on leaf surface microbes. If detrital DOM is of primary importance to the microheterotrophs, then the $\delta^{13}C$ difference between leaf litter species should be represented (statistically de-

tectable) in macroinvertebrate tissue from the different channels. If, however, microheterotrophs are primarily dependent on non-detrital DOM, no difference would be expected between channels in $\delta^{13}C$ values of macroinvertebrate tissue.

Alternatively, in a more natural approach, one could cover a small forested stream with netting from its headwater for a distance downstream to exclude any terrestrial plant litter. The covered section would then be amended with a compensating quantity of litter from a species of terrestrial C_4 plant (such as a marsh grass), which would differ greatly in $\delta^{13}C$ value from the natural litter received by the stream. In this way, the $\delta^{13}C$ value characterizing the source of detrital DOM in this stream section would be changed greatly, while the sources of non-detrital DOM would be unaltered. If detrital DOM was of primary importance to microheterotrophs and consequently to consumers, one would expect a difference in $\delta^{13}C$ value of consumer tissue sampled before and after experimental modification of the detrital DOM source.

A third possible approach would involve transferring stream-incubated leaf litter to the downstream end of a long (i.e. 50 m), darkened, flow-through, in-stream trough. In a darkened trough of this length and with a large bottom surface area relative to flow, one could assume that any non-detrital DOM entering would be utilized before reaching the downstream end. Leaf litter would be added periodically to the darkened trough to the approximate concentrations of detrital DOM available in the stream. The magnitude of any change in microheterotroph activity on leaf litter transferred to the trough (measured before acclimation to the in-trough DOM pool occurs) relative to activity on control litter *in situ* should indicate the relative trophic importance of non-detrital DOM for these microheterotrophs. The unidirectional flow of streams makes them ideally suited for this type of experiment because, in other aquatic habitats, it would be impossible to manipulate concentrations of detrital and non-detrital DOM *in situ*.

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