ALTERATION OF MICROBIAL METABOLIC ACTIVITIES IN ASSOCIATION WITH DETRITUS

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ABSTRACT

Detritus serves as a microhabitat, or microzone, hosting redox gradients, altered microbial metabolism and associated biochemical nutrient transformation processes qualitatively and quantitatively distinct from the ambient planktonic environment. Through the application of tetrazolium redox indicator salts, microautoradiography and microelectrode analyses, it has been shown that distributions of microbial communities and their extracellular deposition products on detrital as well as non-detrital submersed surfaces are patchy in nature, both in terms of physical distribution and associated metabolism. This patchiness promotes the development of steep redox gradients, which in turn lead to diverse microhabitats in which microorganisms, protozoans and invertebrates, all having narrow environmental tolerances, can reside. The compact nature of gradients helps promote diffusive exchange of metabolites, gases and nutrients, thereby maintaining community diversity and structural stability of microzones. Microzones also harbor specific nutrient transformation processes (i.e., nitrogen cycle N_2 fixation, ammonification, nitrification and denitrification) which may otherwise be unfavorable or inhibited in ambient waters.

The promotion, regulation and maintenance of specific nutrient transformation reactions in microzones are all implicated in determining nutrient availability and trophic states of affected aquatic systems.

The importance of particulate organic carbon (POC) in structuring aquatic food chains and regulating micro- and macrobiotic metabolism has long been realized (Pütter, 1909; Krogh, 1930; 1931; Waksman and Carey, 1935; Zobell and Anderson, 1936; Stark et al., 1938; Parsons and Strickland, 1962; Riley, 1963). During the course of these studies defined relationships between submersed surface areas, maintained by particle production and loading, and biotic production have emerged. Furthermore, regulation of nutrient release, uptake and recycling rates has also been linked to qualitative and quantitative particle characteristics of natural waters (Jannasch and Pritchard, 1972; Paerl and Goldman, 1972; Hendricks, 1974; Fletcher, 1979; Kirchman and Mitchell, 1982; Paerl and Merkel, 1982). Despite our appreciation for the trophic importance of non-living POC (detritus), knowledge of the fine structure, associated biotic and resultant chemical alterations characterizing detritus as a dynamic microenvironment has gained momentum only in the past decade, largely in the wake of the First International IBP-UNESCO Detritus Symposium (Melchiorri-Santolini and Hopton, 1972). In this Symposium efforts were made to recognize the physical, chemical and metabolic impacts of detritus on aquatic ecosystems. From these efforts we realized the complex structural, associated microbial and diverse chemical characteristics of detritus (Wangersky, 1977; Silver et al., 1978; Alldredge, 1979; Gowing and Silver, 1983). Throughout the range of aquatic ecosystems considered, a great deal of variability among the above characteristics emerged. Lastly, it was shown that microbial metabolism, including nutrient utilization and release, was altered in the presence of detritus. It has subsequently been shown that particle-associated microorganisms exhibit metabolic activities (including cellular photosynthetic and nutrient uptake rates) distinctly different from those for free floating microorganisms (Paerl and Goldman, 1972; Hendricks, 1974; Fletcher, 1979; Paerl and Merkel, 1982). Such differences also hold true for single species populations ob-

served in attached vs. free floating states. Recent work has repeatedly stressed the existence and importance of surface habitats, or microzones, as sites of altered metabolic activities among microorganisms either attached or associated with detrital particles and other submersed surfaces (Bitton and Marshall, 1980; Savage and Fletcher, in press). In the context of findings reported here, microzones are defined as regions located at the liquid solid interface or in particles where the activities of resident microbial communities lead to environmental conditions distinctly different from the surrounding bulk phase or aqueous media. The term microzone has previously been used by numerous investigators, starting with Nikitin (1973).

As complements to the earlier deployment of electron and fluorescence microscopy, redox indicator salts, microelectrode and microautoradiographic techniques have led to a more detailed and dynamic view of microbial activities and resultant nutrient transformations attributable, and at times unique, to detrital microzones. Previous observational studies, particularly those of MacRitchie and Alexander (1963), Nikitin (1973), Paerl (1973; 1975; 1978), Marshall (1976), Matson and Characklis (1976), Ellwood et al. (1979) and Corpe (1980) have laid the groundwork for more contemporary process-oriented studies designed to better define physical-chemical gradients and resultant promotion or acceleration of specific biochemical nutrient transformation reactions known to exhibit narrow tolerance ranges to ambient oxygen, pH, CO_2 and nutritional states. In summarizing some of the dynamic aspects of microzone formation, Marshall (1976) stressed the importance of diffusive, turbulent and nutrient solubilization alterations in response to initial colonization by microbial populations. This diversification of physical-chemical characteristics leads to microzones which are successively inhabited by microbial, protozoan and invertebrate populations, each of which contributes to, and in turn benefits from, respective impacts on oxygen, diffusive gaseous, metabolite and nutrient gradients. With respect to detrital surfaces, associated microzones can range from less than 10 to several 100 μm in thickness. Recently, both microelectrode (Jorgensen, 1977; Jorgensen et al., 1979; Jorgensen and Revsbech, 1983) and tetrazolium redox salt indicator (Paerl and Kellar, 1978; Paerl and Bland, 1982) studies have demonstrated the existence and ubiquity of redox gradients located within microzones. The oxygen regimes within such gradients serve as diagnostic indicators for the presence and promotion of aerobic, anaerobic and microaerophilic communities known to conduct specific nutrient transformation processes (Cappenberg and Jongejan, 1977; Indrebo et al., 1977; Jorgensen and Revsbech, 1983). The diffusion of nutrients, dissolved gases and metabolites can be readily altered by the excretion of mucilagenous capsules, sheaths and slimes in microzones (Matson and Characklis, 1976; Caldwell and Caldwell, 1978). Currently, the application of relevant established and new technology is revealing the dynamic nature of microzones.

Given the recently-derived knowledge summarized above, an overview of current evidence for diverse and altered microbial and associated microzoan metabolism in detrital microzones is presented. The trophic importance of microzone-mediated metabolism in aquatic ecosystems is also considered.

MATERIALS AND METHODS

Detrital microzones were examined among: (1) decomposing plant particles, typified by macrophyte detritus and colonial phytoplankton, (2) intertidal benthic microbial mats and (3) organic-inorganic aggregates such as silts and sand particles.

Methodology developed and applied by others will be referred to in the results and discussion section. These include the use of microelectrodes, which have proven useful for examining sediments

and microbial mats in marine and freshwater habitats (Jorgensen, 1977; Jorgensen et al., 1979). Methods specifically employed in this laboratory include the application of tetrazolium redox indicator salts and microautoradiography. Short descriptions of each technique and their respective applications are given here.

Tetrazolium Redox Indicator Techniques.—Tetrazolium salts are heterocyclic organic compounds ranging from 200 to 800 MW which irreversibly form insoluble colored crystals, or formazans, upon reduction. In their oxidized states tetrazolium salts are soluble. Due to their relatively low molecular weights they can readily diffuse into most microorganisms and microzones. The reduced formazan is easily detected by light microscopy (either phase contrast or incident illumination). Preliminary work with these salts has shown their use as indicators of reduced microenvironments, including those residing in detrital aggregates, microbial benthic mats, epiphytic and epizoic regions bordering phytoplankton, zooplankton, higher plants and animals, as well as submersed man-made surfaces, such as wooden and concrete pilings and vessel hulls. Numerous tetrazolium salts capable of being reduced through biologically-mediated reactions have been discovered during the past 40 years (Altman, 1972). This has led to a current inventory of approximately 30 useful salts, each exhibiting a specific redox potential required for reduction to formazan crystals. Microbial physiologists have previously employed the following salts as indicators of biological reduction in algae and bacteria (Altman, 1972) (salts are listed in order of increasing redox potential): (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT), $-0.05V$; 3,3-(3,3-dimethoxy-4,4-biphenylene)-bis-2,5,-p-nitrophenyl-SH-tetrazolium chloride) (TNBT), $-0.02V$; 3,3-(3,3-dimethoxy-4,4-biphenylene)-bis-(2-p-nitrophenyl-5-phenyl-2H-tetrazolium chloride) (NBT), $-0.01V$; 3-α-naphthyl-2,5-diphenyl-2H-tetrazolium chloride (TV), $+0.29V$; 2,3,5-triphenyltetrazolium chloride (TTC), $+0.40V$. All five salts were employed in current studies. NBT, TNBT, TTC and TV were dissolved in distilled water as 1% w/v stock solutions maintained in dark bottles (to avoid photoreduction) at 4°C. INT was initially dissolved in a small quantity of 95% ethyl alcohol, and then diluted to 1% w/v with distilled water. Final experimental concentrations were 0.01% w/v for INT, TTC and TV and 0.005% w/v for NBT and TNBT. Following tetrazolium additions freshwater and marine samples were incubated on a slowly (15 rpm) gyrating shaker under both illuminated (300 $\mu E \cdot m^{-2} \cdot S^{-1}$ cool white plus gro-lux fluorescence) and dark conditions at sampling temperatures for periods lasting from 10 min to 3 h. Tetrazolium reduction was stopped by a 3% v/v buffered (pH 7.5) glutaraldehyde addition. Glutaraldehyde-fixed samples were then observed by either incident light or phase contrast microscopy using a Zeiss model K research-grade microscope at magnifications ranging from 200 to 1,000×. Care was taken to minimize disturbance of particulate matter observed by microscopy. In some cases samples were directly placed on microscope slides (illuminated or darkened) followed by tetrazolium salt incubation, glutaraldehyde fixation and observation. Formazan deposition was recorded photographically using Ilford pan-F fine grain black and white film developed with Agfa-Rodinal fine grain developer.

Microautoradiographic Methods.—Material examined for tetrazolium reduction was also utilized for microautoradiographic detection of nutrient assimilation in microzones. Assimilation of the following nutrients was observed; $^{33}PO_4$, $^{14}CO_2$ and a ^3H labeled L-amino acid mixture. $^{33}PO_4$ was obtained in carrier-free form from New England Nuclear Corp. It was added at 5 μCi per 100 ml of sample. $^{14}CO_2$ was added at 6.25 μCi per 100 ml as $NaH^{14}CO_3$, obtained at 58 mCi·m mol^{-1} from Amersham Radiochemicals. A ^3H uniformly labeled L-amino acid mixture, having a specific activity of 210 mCi·m mol^{-1}, was obtained from Amersham Radiochemicals. It was added at 10.5 μCi per 100 ml sample. Isotope assimilation experiments were conducted in a fashion similar to tetrazolium salt incubations; the only exception was the use of longer incubation periods, which lasted up to 24 h.

Following incubation, small volume samples (1–5 ml) were immediately filtered at gentle filtration pressures (100 Torr) onto 0.45 μm porosity 25 mm diameter HA Millipore filters, followed by a double rinse (10 ml each) of distilled deionized water. All filters were then immediately immersed in liquid N_2 for freeze fixation according to Paerl (1984). This technique assures optimal structural integrity of detritus while minimizing fixed isotope loss. All freeze-fixed filters were freeze dried in preparation for microautoradiography (Paerl, 1984). In the case of $^{14}CO_2$ assimilation experiments, all filters were fumed in an HCl atmosphere for 20 min to eliminate abiotically-precipitated ^{14}C.

All filters were stained in a 2% w/v erythrosin-B 5% w/v phenol mixture for 2 h, destained in distilled water for 1 h and air-dried (Paerl, 1978). Dried filters were mounted, face up, on clean microscope slides by applying glue to the edge of each filter. Slides were then passed over the mouth of a 600 ml beaker containing approximately 20 ml of boiling acetone. Filters are rendered transparent within 2 sec while forming a firm bond to the slides. Microautoradiographs were prepared in complete darkness by dipping each slide into molten (40°C) Kodak NTB-2 nuclear track emulsion diluted 1:1 with distilled water. This yields thin layer grain density autoradiographs (Paerl and Stull, 1979). Dipped slides were dried on staining racks for 30 min and packed in light-tight slide boxes fitted with packets of silica gel desiccant. Exposures of slides were conducted from 1 to 10 days, depending on magnitudes of localized isotope assimilation. Following development (Paerl and Stull, 1979) microautoradiographs
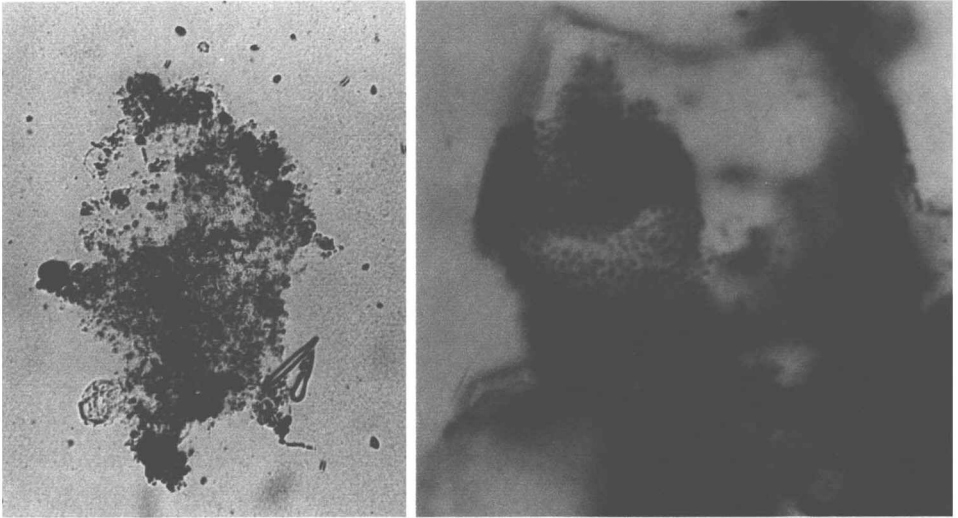
Figure 1.   (Left) Deposition of reduced INT formazan crystals in a detrital aggregate freshly sampled from Bogue Sound, N.C. (32 ppt salinity). Specific regions of the aggregate revealed strongly reduced conditions, while other regions remained free of formazan deposition. This sample was incubated for 1 h under illuminated conditions. Control (formalin-killed) aggregates failed to reveal formazan deposition under either illuminated or dark conditions.

Figure 2.   (Right) High-magnification view of INT formazan deposition in regions bordering sand grains sampled from an intertidal marine benthic region on Shackleford Island, N.C. Such sand grains serve as the colonizing substrate for bacteria and cyanobacteria making up a microbial mat. Note both the individual bacteria coating a sand particle as well as interstitial spaces between particles reveal reduced conditions.

were observed with either bright field or phase contrast light microscopy at magnifications ranging from 200 to 1,000×. Microphotographs were recorded on Ilford pan-F film.

RESULTS AND DISCUSSION

Since oxygen conditions are of central importance with respect to locations and biochemical characteristics of microzones, results will focus on oxygen gradients and their impacts on the establishment and modification of nutrient cycling processes.

Observations of tetrazolium (formazan) deposition are a useful initial indicator of potential microbial habitats supporting reduced conditions. Using this technique one can readily observe reduced microzones bordering decomposing *Spartina* particles, organic detrital aggregates as well as inorganic-organic matrices, such as sand grains mixed with marsh-derived decomposition products (remnants of macrophytes, benthic periphyton, invertebrate fecal pellets). Such microzones are normally indicative of extensive microbial surface growth. In general, however, formazan deposition in these regions is not exclusively confined to bacteria but also can be found in intercellular detrital spaces occupied by mucilagenous excretions and organic deposits (such as remnants of previous microbial, protozoan and invertebrate inhabitants) (Figs. 1 and 2). Hence, even though reduction of tetrazolium salts is initiated by intracellular biochemical processes, one overall effect of microzone formation is the creation of low extracellular oxygen tensions, as demonstrated by formazan deposition in detrital "patches" not containing

bacteria. In this manner strong oxygen gradients can be established in microzones, making them potentially feasible for nutrient transforming processes reliant on reduced conditions, even when ambient waters support full oxygen saturation. Among decomposing algal aggregates the establishment of bacterial epiphytes on surfaces as well as within such aggregates is normally accompanied by rapid deposition (both intra- and extracellularly) of a variety of reduced formazan crystals, including those of INT ($E_h = 0.05V$), NBT ($E_h = -0.01V$), TV ($E_h = +0.29V$) and at times TTC ($E_h = +0.40V$). Reduced microzones typically reveal non-uniform distributions around or in detrital particles (Fig. 1). The establishment of reduced zones is often confined to patches; specifically those patches harboring extensive microbial, protozoan and (at times) invertebrate populations (Figs. 1 and 2).

The combined effect of numerous reduced patches can lead to the formation of layered oxygen gradients in some microzones. A notable example of such a chain of events are the benthic microbial mats attached to sand particles in intertidal marine lagoons. One such habitat is located on Shackleford Island, one of a series of barrier islands separating North Carolina's sounds from the Atlantic Ocean. The mats are generated from 10 to 50 μm thick microzones bordering sand grains (Fig. 2). Initially, microzones are established by heterotrophic bacteria, cyanobacteria and diatoms accompanied by excreted fibrillar and mucilagenous organic deposits. Reduced patches are evident at this stage of microzone development. Following the establishment of this pioneer community, diversification occurs along algal, bacterial and protozoan lines. At this stage the microzone-mat varies from approximately 100 to 500 μm in thickness. On the mat's surface (exposed to intertidal water movement) the cyanobacterial component becomes partitioned between a photosynthetically-active upper layer of the filamentous non-N_2 fixing genera *Lyngbya* and *Oscillatoria*, while 50–100 μm below it a distinct layer of the filamentous N_2 fixing genus *Microcoleus* dominates. Underneath the *Microcoleus* layer the anaerobic purple photosynthetic bacterium *Chromatium* forms a thin 15–20 μm layer. This layer is particularly evident during mid- to late summer months when organic deposition due to extensive cyanobacterial and bacterial growth in the upper portion of the mat is being decomposed, creating oxygen-depleted microzones potentially utilizable for obligate anaerobes such as *Chromatium*. The overall result of intense photosynthetic production of organic matter in the upper layers of the mat coupled with heterotrophic decomposition of this organic carbon source has led to extreme oxygen gradients in the mat microzone; using microelectrode analyses we have measured dissolved oxygen ranging from 250% saturation in the upper portions (non-N_2 fixing cyanobacteria) to anaerobic conditions only 200 μm down into the mat (the interface between *Microcoleus* and *Chromatium*) (Fig. 3). Within the *Microcoleus* layer, which lies in a transitional dissolved O_2 concentration zone, highly reduced patches exist. These patches are apparently attributable to concentrations of heterotrophic bacteria, which through their active metabolism create O_2-deficient regimes. *Microcoleus* is commonly observed with a bacterial coating surrounding the bundles of filaments in which it is naturally found. Similar bacterial associations have been found among naturally-occurring bundles of the N_2 fixing marine cyanobacterium *Trichodesmium* (Carpenter, pers. comm.). In freshwater habitats bacterial growth is often specifically confined to the N_2 fixing sites (heterocysts) of the filamentous cyanobacteria *Anabaena* and *Aphanizomenon* (Fogg et al., 1973). Paerl and Kellar (1978) have shown such associations to be instrumental in promoting N_2 fixation among the above cyanobacteria, supplementing structural modifications offered by heterocysts. The mechanism promoting N_2 fixation involves localized O_2 re-
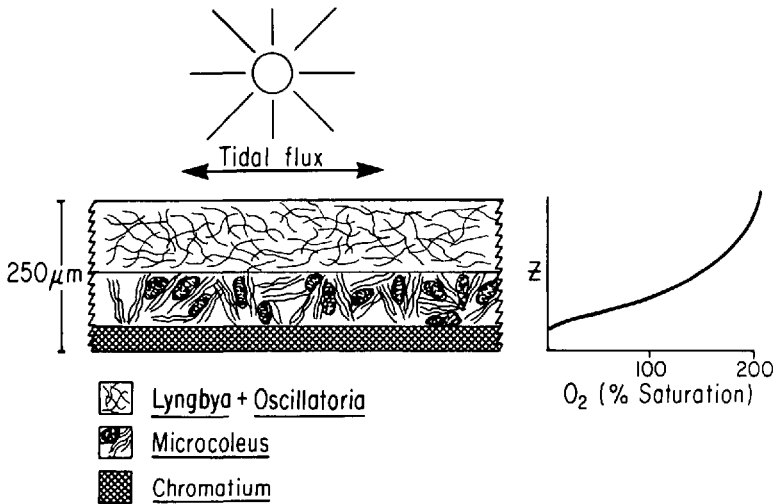
Figure 3.   A profile of the Shackleford Island microbial mat, showing distinct layering of cyanobacterial and photosynthetic bacterial communities as well as dissolved oxygen conditions typifying various regions of the mat. Dissolved oxygen gradients were measured through the use of microelectrodes.

moval (around heterocysts) by actively respiring bacteria (Fig. 4), thereby minimizing excessive oxygen tension near the N_2 fixing loci. The same may be true for marine N_2 fixing cynaobacteria, particularly those genera lacking protective heterocysts (*Microcoleus, Trichodesmium*). Indeed, when *Microcoleus* is physically separated from the Shackleford mat community and rendered free of associated bacteria (bacteria were removed by mild sonication and extensive washing), N_2 fixing activities rapidly declined (Table 1). Photosynthetic activities, per unit chlorophyll *a*, were relatively unaffected (Table 1), indicating that *Microcoleus* remained viable following this treatment. When the same *Microcoleus* population was allowed to be recolonized by the natural bacterial flora, N_2 fixation rates, per unit chlorophyll *a*, recovered to near-original rates (Table 1). Furthermore, separated *Microcoleus* bundles maintained depressed N_2 fixation rates in cultures maintained by shaking or stirring. When these cultures were allowed to remain in a non-turbulent state light-mediated chlorophyll *a* specific N_2 fixation rates rapidly increased (Fig. 5). The most likely explanation for alterations (decreases) in *Microcoleus* N_2 fixation rates either by turbulent mat disturbance or removal of associated microflora in that this cyanobacterium is highly dependent on the existence of microzones, containing microbially-mediated oxygen gradients, for optimizing N_2 fixation. Microbial mats and detrital aggregates offer such an environment.

In systems where nitrogen has repeatedly been shown to be a growth-limiting nutrient for both planktonic and benthic algae, microzone promotion of N_2 fixation may play a crucial role in optimizing overall microbial biomass production. The sounds and coastal waters of North Carolina typify these conditions. Microzone dissolved oxygen gradients, as witnessed in mat communities, allow several aerobic and anaerobic processes, not found in close proximity in planktonic systems, to operate in a closely coupled fashion on surfaces. For example, environmental oxygen requirements for ammonification, nitrification, denitrification and
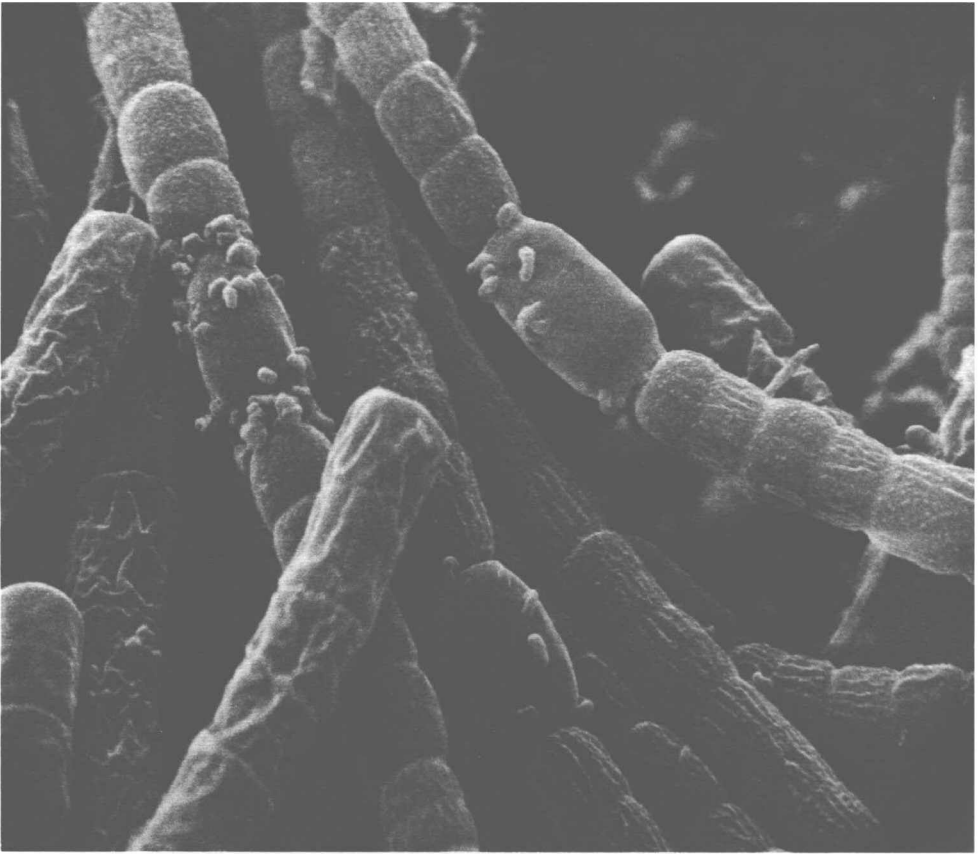
Figure 4. Bacterial populations specifically associated with the heterocysts of the N_2 fixing filamentous cyanobacterium *Aphanizomenon flos aquae*. Heterocyst-associated bacteria revealed high rates of heterotrophic activity (both amino acid and sugar uptake) as well as high respiratory rates (as evidenced by INT, NBT, TV reduction to respective formazan crystals in regions where bacterial populations were observed). This scanning electron micrograph was taken from Paerl and Keller (1978).

N_2 fixation can all be met within a 20–300 μm thick mat microzone (Fig. 3). Previous work with marine and freshwater particulate matter in oligotrophic habitats has pointed to the importance of surface area represented by such particles as biostimulatory to nitrification (Carlucci and Strickland, 1968; Paerl al., 1975; Kholdebarin and Oertli, 1977). Recently, Schropp and Schwartz (1983) provided evidence from the Tropical North Pacific Ocean and Caribbean Sea implicating particle-associated bacteria in the water column as being responsible for observed denitrifying activities. In a study of sources and sinks of nitrite in Chesapeake Bay, McCarthy et al. (1984) found unexpectedly high rates of both nitrification and dissimilarity NO_3^- reduction to NH_4^+ in the water column. These data also strongly suggest the presence of reduced and anoxic microzones harboring these nutrient transforming processes in the oxygenated water column. Similar examples exist in soils, where specific clay and sandy soil types greatly stimulate both ammonification and nitrification (Stotzky, 1972; Kunc and Stotzky, 1980). In concert, the aquatic and soils data implicate the establishment of microzones
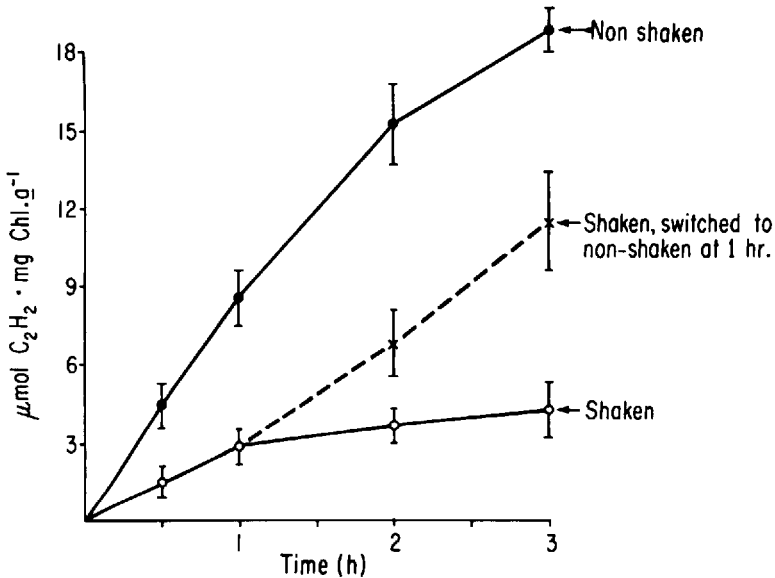
Figure 5. Acetylene reduction characteristics of *Microcoleus* spp. bundles isolated from the Shackleford Island microbial mat and subsequently maintained in filtered seawater. Assays were conducted within 3 h of initial sampling, under 300 $\mu E \cdot m^{-2} \cdot S^{-1}$ PAR illumination. Triplicate samples were either shaken or incubated under static (non-shaken) conditions. At 1 h, a triplicate set of shaken samples was switched to and maintained on non-shaken conditions.

generating elevated concentrations of metabolic intermediates as the biostimulatory factor responsible for the observed enhancement of nitrogen transformation reactions. When considering ammonification and nitrification on a microzone scale, end products of one process (decomposition) should readily diffuse to regions where they are required by another (nitrification). Organic particle surfaces undergoing decomposition represent greatly elevated sources of ammonia when compared to planktonic environments, particularly in oligotrophic ecosystems (Shanks and Trent, 1979). Microzone-oriented nutrient transformation processes, such as discussed above should optimize not only efficient nutrient cycling, but also lead to increased fertility of ambient waters.

Table 1. N_2 fixation (acetylene reduction) and CO_2 fixation (using the ^{14}C method) in *Microcoleus* spp. separated from the Shackleford Island (N.C.) microbial mat. Following physical separation, bundles of filaments were maintained in filtered seawater. Some bundles were rendered bacteria-free by mild sonication, followed by extensive washing in filtered seawater. Triplicate samples were incubated for 3 h on a slowly rotating gyratory shaker under both 300 $\mu E \cdot m^{-2} \cdot S^{-1}$ PAR illumination and darkness. All rate measurements are expressed as μmol per mg chlorophyll *a* per h

Treatment	C_2H_2 reduction		CO_2 fixation	
	Light	Dark	Light	Dark
Control (untreated bundles)	8.2 ± 0.6	2.1 ± 0.3	120 ± 16	ND*
Bacteria-free bundles	2.7 ± 0.4	1.4 ± 0.5	112 ± 21	ND
Bundles recolonized by bacteria	6.3 ± 0.8	2.5 ± 0.4	135 ± 31	ND

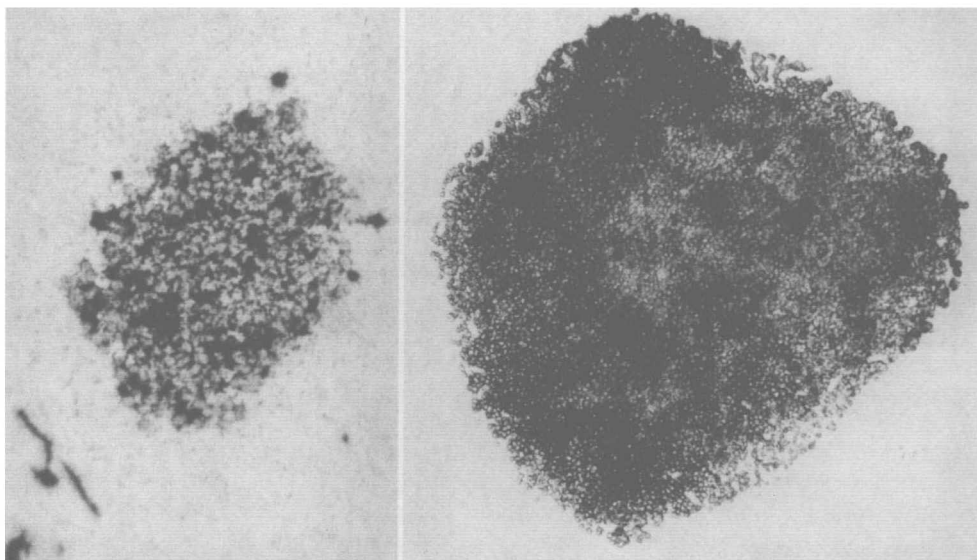* ND = Non-detectable rates. Standard error values, based on triplicate measurements, are given.

Figure 6. (Left) Microautoradiograph of ^3H amino acid assimilation in a decomposing *Microcystis aeruginosa* aggregate. Both detrital assimilation patterns as well as assimilation by free floating bacteria can be observed in this sample. ^3H incorporation can be seen as dense black patches of reduced silver grains in this photomicrograph. (Right) Tetrazolium (TV) reduction patterns observed in a decomposing *Microcystis aeruginosa* aggregate. Dense patches represent highly reduced regions. This sample was incubated for 1 h under illuminated conditions. Formalin-killed controls revealed no significant TV reduction.

At the sediment water interface and adjacent layers of marine and freshwater sediments the abilities of specific microbial populations to optimize sulfur, iron, manganese and carbon transformations are also closely dependent on the establishment and structural integrity of microzones typified by steep redox gradients (Jorgensen, 1977; Reeburgh and Heggie, 1977; Indrebo et al., 1977; Cappenberg and Jongejan, 1977; Strohl and Larkin, 1978; Nealson and Ford, 1980; Jorgensen and Revsbech, 1983). Similarly, the epilithic microbial communities in streams reveal greatly altered (enhanced) nutrient cycling characteristics when compared to the planktonic overlying waters (Goldman and De Amezaga, 1975; Geesey et al., 1978; Haack and McFeters, 1982).

Direct examples of altered (enhanced) metabolism in detrital microzones can be obtained from observations on various decomposition stages of algal aggregates. Through the use of both tetrazolium reduction (formazan deposition) and microautoradiography, zones harboring actively metabolizing bacteria and protozoans are readily visible with either technique; such zones also coincide extremely well in comparisons of both techniques. Both $^{33}PO_4$ and ^3H amino acid assimilation were followed as indicators of enhanced heterotrophic activity in decomposing aggregates of the cyanobacterium *Microcystis aeruginosa* in the Neuse River Estuary, N.C. It was evident from microautoradiographic observations that microbial metabolism is patchily distributed (Fig. 6a); tetrazolium reduction exhibits similar patterns (Fig. 6b). To large degrees tetrazolium reduction spatially coincides with loci of enhanced bacterial biomass present in patches. It remains unclear how such patches become established during detrical formation, since the

substrate available for colonization (in this case decaying *Microcystis* cell) appears rather homogeneously distributed throughout the colonies. However, microautoradiographic examinations of $^{14}CO_2$ incorporation into photosynthetically-active cells in colonies also revealed patchiness, with specific regions of colonies having from 5 to 20 times the photosynthetic activity of others. The dimensions of photosynthetically-active patches roughly coincide with those showing enhanced ^3H amino acid assimilation and tetrazolium reduction by associated bacteria. These concurrent findings have led us to test the possibility that localized enhanced photosynthetic production may lead to enhanced heterotrophic assimilation of organic compounds in patches residing in detrital microzones.

During our microscopic examinations of detrital aggregates it has become increasingly evident that protozoan and (occasionally) microinvertebrate inhabitants of detritus are integral components of microbial metabolism and resultant detrital nutrient cycling processes. Decomposing algal aggregates offer some insight into the potential roles that protozoans may play in establishing and maintaining detrital microzones. Ciliated, flagellated and amoeboid protozoans commonly inhabit *Microcystis* as well as other algal colonies. Flagellates and ciliates are often found grazing bacterial epiphytes on the periphery of such colonies (Fig. 7a). *Amoeba*, on the other hand, penetrates the colonies and can be seen engulfing internal portions of colonies, ranging from a few to 10–15 μm in diameter. Intact *Microcystis* cells are often observed inside actively feeding *Amoeba* (Fig. 7b). Consumption of *Microcystis* cells by *Amoeba* can reach such a high level of activity that these protozoans appear round to ovoid, filled with *Microcystis* cells. This minimizes the extrusion of pseudopodia. When *Microcystis* cells have been digested, pseudopodial formation, and subsequent feeding on internal segments of colonies, continues. Eventually active *Amoeba* feeding leads to cavities devoid of *Microcystis* cells but which are rapidly filled with dense bacterial populations (Fig. 7c). Enhanced ^3H amino acid assimilation rates as well as tetrazolium reduction are associated with these bacterial patches. Clearly, several biotic mechanisms are therefore feasible in explaining the patchy nature of microzone formation. The coupling of active photosynthetic zones to active heterotrophic zones as well as the coupling of protozoan feeding to microbial activities are two distinct possibilities.

The close coupling of photosynthetic, primary heterotrophic (bacterial) and secondary heterotrophic (protozoan) activities appears heavily implicated in creating oxygen gradients in microzones responsible for altering and, in certain cases, supporting specific biochemical nutrient transformations. Detrital metabolic coupling between microbial photosynthetic autotrophs and heterotrophs is illustrated in Figure 8a, while the further interaction of protozoan grazers is diagrammed in Figure 8b.

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Figure 7. a. (Upper) Photomicrograph of detrital aggregate freshly sampled from the Neuse River (freshwater), N.C. Ciliated protozoans, such as the organism shown here, actively grazed bacteria attached to the periphery of aggregates. b. (Center) *Amoeba* grazing inside a decomposing *Microcystis aeruginosa* aggregate freshly sampled from the Neuse River (freshwater). Engulfed *Microcystis* cells can be seen in one of the *Amoeba*. c. (Lower) During protozoan (largely *Amoeba*) grazing inside *Microcystis* aggregates, cavities devoid of *Microcystis* result. These cavities are subsequently occupied by both filamentous, coccoid and rod-shaped bacteria, as shown in this photomicrograph. Actively feeding *Amoeba* and ciliates can also be seen in these cavities.
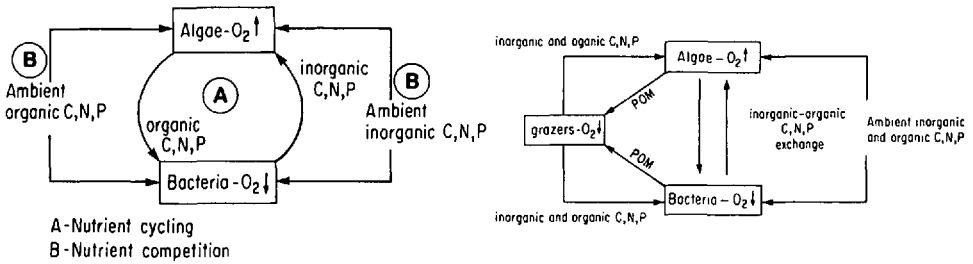
A-Nutrient cycling
B-Nutrient competition
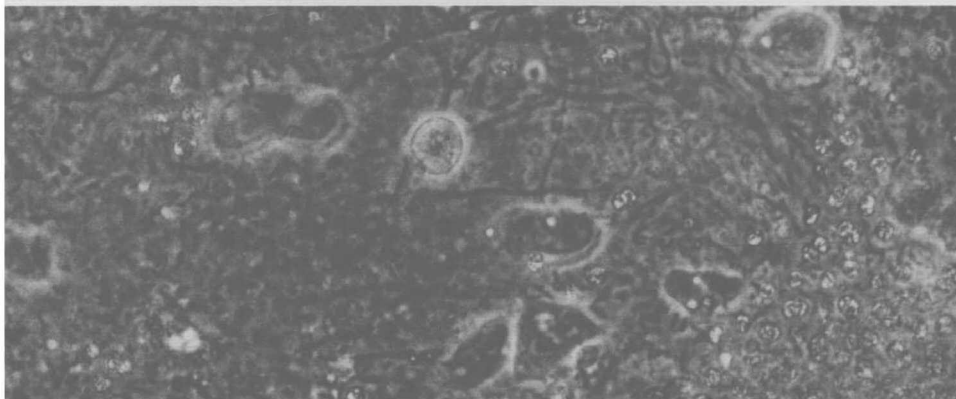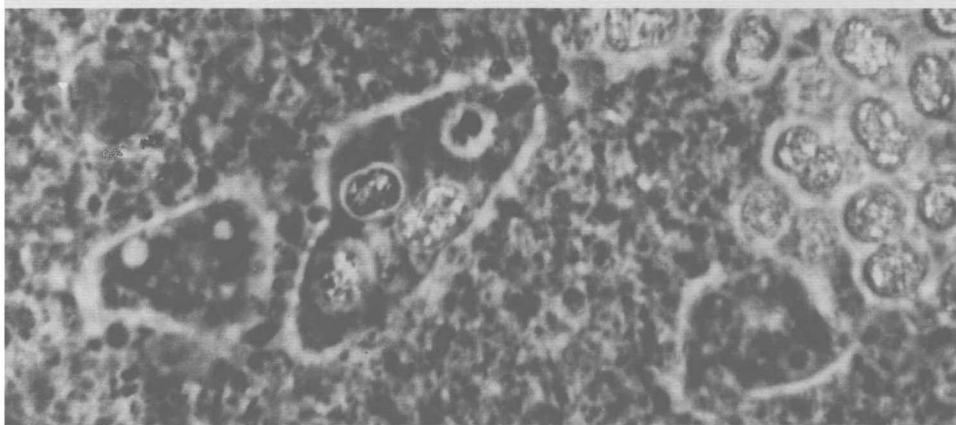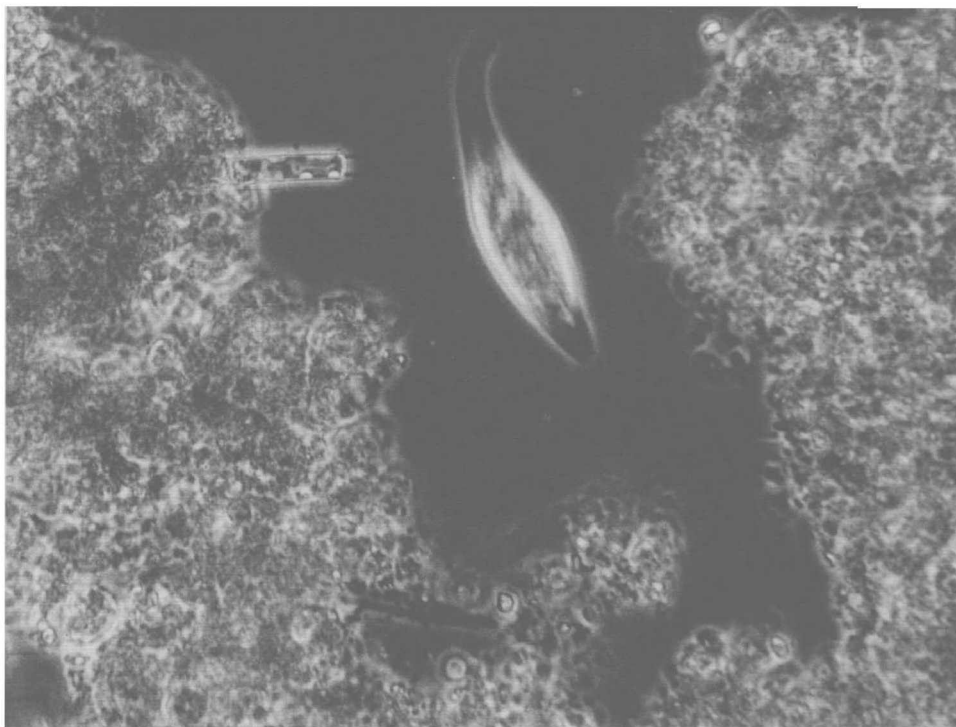
Figure 8.   a. (Left) Detrital metabolic coupling scheme for the major nutrients carbon, nitrogen and phosphorus. Sources of microbial photosynthetic oxygen enrichment and utilization are indicated. Potential nutrient competition and cycling interactions are distinguished. b. (Right) Incorporation of microzoan grazers into a detrital metabolic coupling scheme for major nutrients. Sources and sinks of oxygen are also illustrated.

Because groups of autotrophs and heterotrophs can interact within the small spatial redox gradients characterizing microzones, nutrient and metabolite exchange via diffusion can actively maintain individual, and in certain cases, radically-different, nutrient transformation processes not feasible in ambient waters. In ambient waters oxygen gradients required by (metabolically) specialized microorganisms exchanging metabolites or end products are periodically destroyed by turbulent processes, including laminar flow, wind mixing and convective mixing. Exceptions to this are meromictic lakes, where a permanently stratified monomolimnion harbors specialized (in terms of oxygen and nutrient requirements) microorganisms which are able to orient themselves at the aerobic (epilimnetic)-anaerobic (permanently stratified hypolimnion) interface. These organisms enjoy similar benefits as those located in the strong but stable oxygen gradients present in detrital microzones. They obtain highly reduced electron donors and nutrients by diffusion from nearby anaerobic sections of this gradient, while benefitting from abundant energy sources (either as fixed carbon or light) in the oxygenated portions of the gradient. The structural stability of oxygen gradients in detrital microzones no doubt offers a great deal of opportunity to diverse metabolic types of microorganisms. Even periodic breakdowns of microscale gradients interrupts diffusive nutrient exchange processes, physical contact among partners exchanging metabolites, and oxygen regimes required by those partners having narrow oxygen tolerance ranges.

On a large-scale perspective, lake, stream or oceanic microzone formation serves numerous crucial functions: (i) it allows specific oxygen-sensitive or oxygen-requiring processes to exist in ambient waters supporting dissolved oxygen concentrations normally unfavorable for such processes; (ii) it promotes specific reductive (O_2 sensitive) processes, important for the maintenance of optimal production of biomass, to operate in oxygenated environments (N_2 fixation, Fe and PO_4^{-3} solubilization, ammonification, nitrification as examples); (iii) spatially, the processing and recycling of metabolites and nutrients is made more efficient by the physical-chemical conditions present in microzones (steep concentration gradients over small distances as opposed to a more homogenously dispersed low concentration condition in macroscale mixed ambient waters); (iv) microzones offer habitats for grazers of microorganisms such as protozoans, rotifers and other invertebrates, whose food resources might be scarce and exceedingly growth-limiting in the ambient planktonic environment.

CONCLUSIONS

Although development of qualitative and quantitative assessment techniques is still in a stage of infancy, preliminary evidence presented here and elsewhere point to the importance of liquid-solid interfaces, and specifically microbial microzones, as major sites of nutrient transformation, nutrient generation and biomass production. In addition, microzones allow specific biochemical redox reactions unfavorable in the ambient macroenvironment to proceed on particle surfaces and in non-turbulent planktonic interfaces (metalimnia of meromictic lakes). In particular, it can be shown that major nitrogen nutrient generation (N_2 fixation) and transformation (ammonification, nitrification and denitrification) reactions are altered (largely accelerated) in microzone environments. In marine and freshwater systems, where growth is regulated by nitrogen availability, the formation and proliferation of detrital and benthic microenvironments promoting new sources of nitrogen input (N_2 fixation) are likely to have an impact on determining trophic states and nutrient dynamic characteristics of those systems.

The ways in which detrital microzones become established and maintain structural and functional integrity are poorly understood. It can be shown that both autotrophic and heterotrophic activities are patchily distributed in detrital particles and in epiphytic communities. Patchy distribution of such metabolic activities, in all likelihood, lead to extensive and steep redox gradients. Such gradients help maintain patchy microenvironments, where successional layers of metabolically-distinct microorganisms orient themselves to locate and fulfill their optimal growth requirements.

Microbial grazers, specifically amoeboid, ciliated and flagellated protozoans, as well as small invertebrates (rotifers, crustacean zooplankton and larval forms of higher animals) also have a profound impact on microzone formation. It can be shown, through currently employed techniques, that grazing activities of *Amoeba* lead to low oxygen patches in microzones (through the localized removal of primary producers coupled with stimulation of bacterial growth). Furthermore, microzoans such as *Amoeba,* and a variety of flagellates and ciliates, appear to stimulate microbial heterotrophic and autotrophic activities in the vicinity of their grazing microenvironments. It would appear likely that microzoan release of algal and bacterial nutrients, coupled to efficient utilization of release products by autotrophic and heterotrophic algae and bacteria, help explain such observations.

Nutrient and metabolite exchange are greatly enhanced in microzones because steep gradients of the above substances are assured over time and space in such habitats. By contrast, in ambient waters such physical structuring of the water column is seldom observed, leaving dilute macrogradient conditions in such particle-free planktonic environments. As a result, numerous biochemical processes reliant on compact, steep oxygen and nutrient gradients are eliminated in planktonic environments while they are allowed to flourish in detrital microzones.

Microzones, such as detrital aggregates, benthic microbial mats and submersed fouling layers tend to perpetuate and proliferate themselves through structural stability offered by microbial excretions (mucilagenous capsules, slimes and fibrillar excretions) which act as adhesives, maintaining aggregate and mat materials. Hence, through optimization of autotrophic and heterotrophic production processes stability is introduced and maintained in microzones.

We have only recently become aware of the dynamic nature of detrital microenvironments, indicating the crucial roles such environments can potentially play in determining nutrient availability, cycling and resultant trophic states of

affected aquatic ecosystems. Major technological obstacles still need to be overcome before we can adequately assess the quantitative importance and roles that microzones play in regulating lake, river and oceanic productivities. Because of the small-scale phenomena being investigated, novel indicator, microprobe and imagery (including laser, microspectrophotometry and microfluorometry) techniques will need to be developed and modified for routine application in attaining the μm-scale resolution necessary for qualitative and quantitative assessments of microzone processes and their impacts on the aquatic environment.

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