27. R. H. Dott, *Science* **166**, 874 (1969).
28. W. Hamilton, *Geol. Soc. Amer. Bull.* **72**, 1307 (1961); D. G. Moore and E. C. Buffington, *Science* **161**, 1238 (1968); R. L. Larson, H. W. Menard, S. M. Smith, *ibid.*, p. 781.
29. Samples taken from the Yakima Basalt, above the unconformity with Picture Gorge Basalt on the Hat Point track [see (*21*)].
30. This lava, sampled in 1964 at the base of the section, is poorly exposed and dipping to the south, suggesting the presence of a local structure or perhaps unconformity. The latter is not indicated, however, in the regional surface geological map [A. C. Waters, *U.S. Geol. Surv. Misc. Geol. Invest. Map I-556* (1968)]. The three analyses for the lava all provide ages in excess of 16 million years, suggesting a possible time gap in this section.
31. A. K. Baksi, D. York, N. D. Watkins, *J. Geophys. Res.* **72**, 6299 (1967).
32. The fieldwork was supported by NSF grant GF 76, as part of a cooperative program between the United States and Japan. A.K.B. was supported by a postdoctoral fellowship from the National Research Council of Canada while this research was being completed.
* Present address: Department of Geology, Carleton University, Ottawa, Ontario, Canada.
21 December 1972; revised 14 February 1973

## Detritus in Lake Tahoe: Structural Modification by Attached Microflora

Abstract. *Readily identifiable groups of microorganisms present on nonliving particulate organic matter (detritus) in the upper waters of Lake Tahoe are attached in specific ways and appear responsible for detrital aggregation. This microflora is associated with active heterotrophic metabolism, but deeper waters possess little detrital microflora and little heterotrophic activity.*

An appreciable pool of organic carbon, nitrogen, and phosphorus is present in suspended matter in lakes. The mean ratio of dissolved to particulate organic carbon is approximately 10 : 1 (*1*). Lake Tahoe, in east California and west Nevada, which has ultraoligotrophic offshore areas not yet affected by man's activity in the watershed (*2*), has ratios of dissolved to particulate organic carbon well below the 10/1 norm. The amount of carbon, nitrogen, and phosphorus in particulate form is a large fraction (20 to 40 percent) of the total standing stock. Due to land disturbance and resultant siltation as well as increased algal growth of both planktonic and attached forms, inputs of particulate organic carbon are increasing. Little is known about the possible use of this material by microorganisms. A basic question persists in the study of microbial utilization of detritus: Do certain microorganisms (bacteria plus fungi) attach to detritus?

Most investigators agree that attachment is common (*3, 4*). Surprisingly, there is little evidence that conclusively demonstrates attachment to detrital matter. Some workers have provided evidence by using phase and ultraviolet light microscopy to substantiate microbial attachment (*3*). The use of stains in light microscopy (*5*) gives results which are difficult to interpret since stains often fail to differentiate between microbes and nonliving material. Autoradiography reveals uptake of organic carbon associated with detritus (*6*), but it is difficult to see cells or mycelia associated with the uptake. Freeze-etch electron microscopy (*7*) and transmission electron microscopy (*8*) yield excellent resolution and have provided workers with detailed photographs of detritus. But the depth of field is limited with the latter technique and attachment to particles is difficult to demonstrate.

Scanning electron microscopy (SEM), in combination with routine sampling and fixing techniques, was therefore employed in order to overcome limitations in resolution and depth of field.

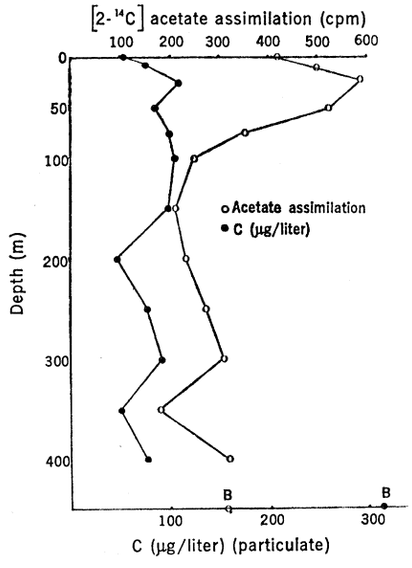


Fig. 1. Relation between heterotrophic activity and particulate carbon in a deep-water profile. Lines are not drawn to the bottom samples, labeled *B*, because of the striking differences in the carbon content of these samples compared to the water column. The maximum sampling depth is 440 m.

Detritus was examined over a vertical profile (0 to 440 m) which was sampled in the middle of Lake Tahoe during the summer of 1972. Subsamples (100 ml) were filtered and prepared for SEM. Gelman 0.45-$\mu$m Metricel filters (2.54 cm in diameter) were used. These filters proved to be resistant to organic solvents used in later steps and had fairly smooth surfaces when viewed with SEM. Filtration was carried out at gentle vacuum pressures (less than 500 torr). Filters were folded in half and clamped in place with a small piece of aluminum foil, which could be labeled. This step was performed within 1 minute after filtration was completed in order to prevent desiccation. Filters were then fixed in 2 percent glutaraldehyde buffered with 0.1*M* sodium cacodylate. Fixation for 45 minutes at 5°C gave good results. Dehydration was done by stepwise immersion of the fixed material plus filters in increasing concentrations of ethyl alcohol. Filters were allowed to remain in each concentration of absolute alcohol (10, 25, 50, 75, and 100 percent) for 10 minutes. After dehydration the filters were transferred to amyl acetate for at least 4 hours in preparation for critical point drying (*9*). Solvent exchange was from amyl acetate to carbon dioxide.

Small squares (25 mm$^2$) were cut from the dried filters and mounted directly on stubs with a silver base adhesive. The stubs were coated in vacuum with two layers of gold, each 100 Å thick, and viewed with a Cambridge Stereoscan scanning electron microscope.

The same samples were monitored for microbial heterotrophic activity. This has been shown to be a sensitive measurement of relative mineralization rates of lacustrine microbes (*6*). Duplicate subsamples were taken at each depth and transferred to sterile 125-ml darkened Pyrex reagent bottles. A trace amount (10 ng of acetate per liter) of [2-$^{14}$C]acetate was added to each sample. The incubation time was 1 hour in a dark incubator set at sampling temperature. Rates of assimilation of acetate over the 1-hour period were compared for samples taken at all 13 depths. The acetate concentration in the lake varied from 1 to 10 $\mu$g/liter. The values were derived from both gas chromatographic analyses and kinetic plots. Assimilation of acetate has been shown to be largely by bacteria (*6*). Determinations of total particulate carbon were made at each depth. One-liter subsamples were fil-

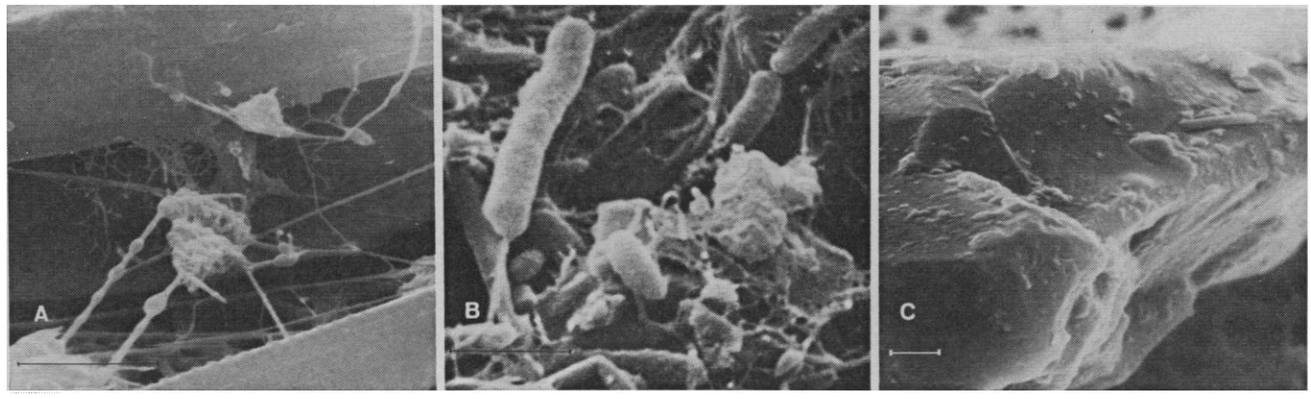


Fig. 2. Observations of detritus by scanning electron microscopy at (A) 20 m (scale bar, 5 μm); (B) 20 m (scale bar, 1 μm); (C) 300 m (scale bar, 1 μm).

tered on Whatman GFC 2.4-cm precombusted filters. Particulate carbon was analyzed by combustion and infrared detection (*10*).

The vertical profile reveals a typical summer distribution of microbial heterotrophic activity (Fig. 1). Changes in rates of assimilation are directly related to temperature changes in Lake Tahoe (*11*). Assimilation rates also reflect growth rates since fungal and bacterial biomass correspond directly with rates of assimilation of organic carbon (*12*). In general, the upper 50 m of the water column show the highest rates of microbial growth.

Vertical changes in heterotrophic activity corresponded to changes in particulate carbon. This relationship was strongest in deep water (below 150 m) where the stimulatory effect of increased near-surface temperatures can be omitted as a variable. In the upper 50 m there were concomitant changes in heterotrophic activity and particulate carbon, but activity per unit of particulate carbon is further amplified by increased temperatures. The amounts of dissolved organic carbon varied little with depth in Lake Tahoe. Between 50 and 150 m the concentration of live phytoplankton cells, which supported few attached microbes, appeared to account for the observed increase in particulate carbon.

Samples for SEM from relatively shallow depths (20 m) showed a close association between bacterial and fungal cells and pieces of detrital material (Fig. 2, A and B). Detritus supporting attached microflora consisted mainly of small pieces of organic and inorganic material, which were usually less than 10 μm in size. Cell sizes for attached bacteria varied from 0.2 μm for small cocci to 3 μm for large bacilli. No attempt was made to separate bacteria and fungi into species, although all the rod-shaped bacteria showed polar flagella and were gram negative. This indicated that the genus *Pseudomonas* was predominant. Fungal mycelia and filamentous bacteria adhered tightly to detritus, and increased filtration pressures did not appear to disrupt or dislodge them. Extensive fungal and bacterial "webbing" was noticed in near-surface samples (Fig. 2A). Fungi seemed to grow into and on the surface of detrital particles. Bacterial cells (Fig. 2B) were surrounded by fine webs which appeared to keep the cells tightly anchored to detrital particles. Free-floating cells with polar flagella did not show weblike structures.

Detrital particles from 75 m were larger (25 to 50 μm) and smoother and showed less microbial attachment. Surface growth on these particles was decreased. Fungal attachment was absent at depths greater than 75 m. Below 150 m detritus showed few attached bacteria (Fig. 2C), but attached remains of microbial cell walls and stalks were observed. Extensive microbial association was noticed only on diatoms, which showed signs of partial decomposition.

Between 150 and 400 m there was little change in the structure of a microbial association with detritus. At these depths detritus was composed of aggregated pieces of mineral (mica, feldspar, quartz) and organic material (remains of zooplankton and plant tissue) all compacted into a smooth pellet with little microbial attachment.

At 440 m, where the sample was taken approximately 20 cm above the bottom, compacted pellets showed a slight increase in attached microorganisms. It should be noted that the Van Dorn sampler with a bottom closing device tends to stir up the uppermost layers of sediment, which are then sampled. Even though microbial growth was noted here it was far less dense than on near-surface detritus. These findings support the results of Menzel and Ryther (*13*) that most of the reactive particulate and dissolved organic matter is decomposed and solubilized in the upper 200 to 300 m of pelagic ocean waters. Detritus settling on the bottom of Lake Tahoe

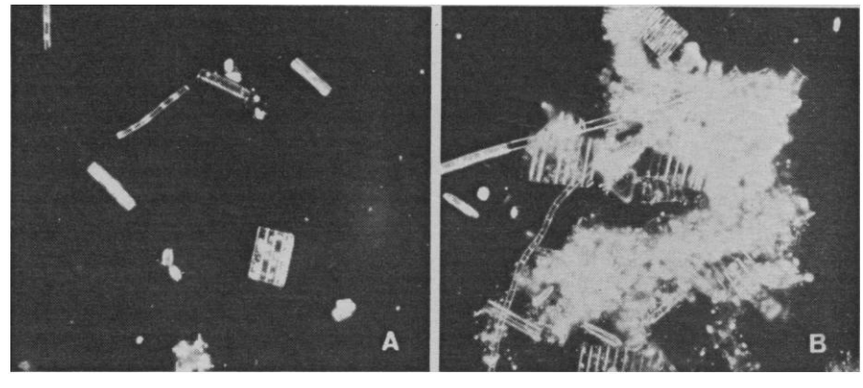


Fig. 3. Phase microscope observations. Morphological changes of detritus sampled from nonsterile dialysis bags after (A) 12-hour and (B) 48-hour incubation periods at 20 m in Lake Tahoe (× 500).

appears to be rather unreactive since it is only attacked by a small number of bacteria.

In a separate study fresh detritus was collected from the mouth of Ward Creek, a tributary presently under intensive investigation (*14*). The detritus consisted mainly of remains of attached algae (periphyton) which had broken loose. The detritus was homogenized and sterilized by autoclaving at 130°C for 15 minutes. It was then divided equally among 150-ml dialysis bags made from tubing tied off and sealed by dipping in hot paraffin at both ends. The bags allowed free diffusion of nutrients and metabolic waste products, but were impermeable to detritus and associated microorganisms. Two sets of dialysis bags were used. One set contained sterile detritus and a 50-ml inoculum of lake water (20-m depth) containing live microorganisms. The other set had similar contents but was sterilized by immersion in 0.001*M* $HgCl_2$ for 12 hours after the bags were sealed. Sterility tests, consisting of plate counts, heterotrophic activity measurements, and microscopic observations, were made on the contents of these bags after a 4-week incubation in Lake Tahoe. Tests showed that microorganisms did not survive sterilization, nor did new cells enter the sealed bags.

Both sets of bags were allowed to incubate at a depth of 20 m in Lake Tahoe (20 m was the depth of maximum heterotrophic activity during the sampling period). Duplicate sterile and nonsterile bags were taken out daily to make observations of the contents by phase light microscopy.

Experiments with in situ dialysis bags gave an indication of the fate of fresh detritus entering from streams. A sequence of light-microscope pictures of the contents of dialysis bags supporting live microorganisms shows aggregation of small detrital particles into larger particles over a period of 3 days (Fig. 3, A and B). After 3 days the particle size in sterilized bags was only 10 percent of the particle size in bags with living microorganisms. Sterile bags showed a slight aggregation of particles. This is possibly due to the attraction of oppositely charged particles or to adsorption of dissolved organic matter on random surfaces (*15*). Aggregation of particles in unsterilized bags may be partly attributable to charge attraction and adsorption, but is largely due to microbial adhesion to particles. Observation of live detritus by SEM shows that particles are not adhering to each other but are trapped by web-like structures associated with bacteria and fungi. Concurrently, particles increase in size and sinking rate.

Structural modification of detritus through aggregation is at least in part attributable to fungal and bacterial growth initiated on small particles. Most of the cementing activity occurs in near-surface waters, where fresh inputs of particulate organic carbon and photosynthetic production of organic carbon are highest. Since the amounts of dissolved organic carbon are relatively low in Lake Tahoe (400 to 500 $\mu$g/liter) the pool of particulate organic carbon represents an important source of nutrition for heterotrophic bacteria and fungi. Exploitation of this pool appears to be extensive in the upper 75 m since both heterotrophic activity and attachment are highest in this zone.

Observations show that bacteria and fungi do not necessarily need stalks or elaborate structural modification for attachment. It appears that bacterial secretions and fungal mycelia cause microorganisms to adhere to particulate substrata. Due to their "stickiness," such microorganisms play a significant role in determining the ecology of detritus.

HANS W. PAERL

*Institute of Ecology,*
*University of California, Davis 95616*

**References and Notes**

1. E. A. Birge and C. Juday, *Ecol. Monogr.* **4**, 440 (1934).
2. C. R. Goldman, G. Moshiri, E. de Amezaga, in *Symposium on Water Pollution Control in Cold Climates*, R. S. Murphy and D. Nyquist, Eds. (Government Printing Office, Washington, D.C., 1972), p. 1.
3. A. G. Rodina, *Limnol. Oceanogr.* **8**, 388 (1963).
4. A. E. Kriss, S. S. Abyzov, M. N. Lebedeva, I. E. Mishistina, I. N. Mitskevich, *J. Bacteriol.* **80**, 731 (1960).
5. Y. I. Sorokin, in *Manual on Methods for Measuring Primary Production in Aquatic Environments*, R. A. Vollenweider, Ed. (Blackwell Scientific, Oxford, England, 1969), p. 133.
6. H. W. Paerl and C. R. Goldman, *Limnol. Oceanogr.* **17**, 145 (1972).
7. H. W. Paerl and R. L. Leonard, in preparation.
8. J. Olah, in *Symposium on Detritus and Its Ecological Role in Aquatic Ecosystems*, U. Melchiorri-Santolini, Ed. (Instituto di Idrobiologia, Pallanza, Italy, in press.)
9. T. R. Anderson, *Trans. N.Y. Acad. Sci.* **13**, 130 (1951).
10. R. Armstrong, C. R. Goldman, D. K. Fujita, *Limnol. Oceanogr.* **16**, 137 (1971).
11. H. W. Paerl, thesis, University of California, Davis (1973).
12. O. Holm-Hansen and H. W. Paerl, in *Symposium on Detritus and Its Ecological Role in Aquatic Ecosystems*, U. Melchiorri-Santolini, Ed. (Instituto di Idrobiologia, Pallanza, Italy, in press.)
13. D. W. Menzel and J. H. Ryther, in *Symposium on Organic Matter in Natural Waters*, D. W. Hood, Ed. (Institute of Marine Science, University of Alaska, Fairbanks, 1970), p. 31.
14. This is part of the Ward Valley watershed study, supported by grant GI-22 from the National Science Foundation–Research Applied to National Need.
15. G. A. Riley, *Limnol. Oceanogr.* **8**, 373 (1963); R. A. Neihof and G. I. Loeb, *ibid.* **17**, 7 (1972).
16. Particular thanks to C. R. Goldman and R. L. Leonard for valuable contributions during the preparation of this manuscript. I am also grateful to R. C. Richards for his assistance in sampling Lake Tahoe. Use of the scanning electron microscope was through the Facility for Advanced Instrumentation, University of California, Davis.

8 January 1973; revised 20 February 1973

## Circadian Rhythms in Neurospora: Spatial Differences in Pyridine Nucleotide Levels

Abstract. *A growing colony of a mutant strain of* Neurospora crassa *had two morphologically distinct areas which were formed as a result of a rhythmic spore-forming (conidiation) process. The total pyridine nucleotide content of these two areas was the same, but the levels of NADH, NADPH, and NADP were lower in the conidiating area, while the NAD level was higher. These biochemical differences in the adjacent areas of a single colony were only found in newly formed areas, and were not a permanent record. It is not known whether these pyridine nucleotide changes are a result of the conidiation process, or whether they are tied more directly to some underlying metabolic oscillation. However, it is speculated that the changes in the levels of these key coenzymes could have far-reaching effects on many areas of metabolism.*

Biological rhythms in fungi are expressed in a variety of ways. In *Neurospora crassa,* the periodic formation of conidia (vegetative spores) occurs in certain mutant strains such as patch (*1*), timex (*2*, *3*), or band (*3*), whereas in other mutant strains (clock) the periodicity can only be seen as changes in the branching frequency of the filamentous hyphae (*4*). In the band (*bd*) strain, the conidiation rhythm has the characteristics of a circadian one, in that the rhythm is not appreciably affected by temperature, can be altered by light, and has a periodicity of approximately 1 day (*3*). In the *bd* strain, both the conidiating phase and the nonconidiating phase can be approxi-