ADVANCES IN MICROBIAL ECOLOGY

Edited by M. Alexander

Volume 4

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Preface

The literature in microbial ecology is growing rapidly. Journals in many countries dealing with microbiology, ecology, environmental sciences, and environmental technology are publishing an ever-increasing number of papers, and these reports are providing microbial ecologists with a wealth of information. This body of data is now so large and the research is published in so many journals and monographs that maintaining an overview of the development of the field grows more difficult. The role of *Advances in Microbial Ecology* thus becomes more obvious with time.

The articles in the present volume encompass an array of topics appropriate to the development of the discipline of microbial ecology. Both terrestrial and aquatic ecosystems are subjects of attention, and a variety of microbiological groups come under review. Furthermore, methodological problems and approaches are not overlooked.

The ecology of protozoa, constraints on their populations, and their role in nutrient cycling and energy flow are considered by J. D. Stout. A unique microenvironment is discussed by B. Norkrans, the surface microlayer of aquatic ecosystems, and Dr. Norkrans presents information on a field that has blossomed in the last few years. The subject of the review by H. S. Lowendorf is the genus *Rhizobium*, a group of bacteria whose importance has grown as the cost of fuel for production of nitrogen fertilizers and ultimately for protein production has increased. Two special terrestrial ecosystems are also considered in the present volume, flooded soils that are common in much of Asia for rice production and Histosols, a related ecosystem in that it is flooded but one that is dominated by organic rather than inorganic materials. I. Watanabe and C. Furusaka are the authors of the former review, and R. L. Tate III is the author of the latter. B. B. Bohlool and E. L. Schmidt present a thorough evaluation of immunofluorescence as a technique for study of the ecology of microorganisms.

The Editorial Board of *Advances in Microbial Ecology* and the sponsors of the series hope to maintain the standards and the direction of the earlier volumes. *Advances* is designed to serve an international audience and to provide reviews written by scientists from various countries. The various reviews that are pub-

lished are selected because they include the basic and applied aspects of microbial ecology and properties of diverse ecosystems and various microorganisms. We believe that the series has been useful in accomplishing this goal, and we express our thanks to our colleagues for their contributions, comments, and suggestions.

M. Alexander, Editor
K. C. Marshall
T. Rosswall
H. Veldkamp

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Microbial Oxidation of Organic Matter of Histosols

Robert L. Tate III

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The Role of Protozoa in Nutrient Cycling and Energy Flow

JOHN D. STOUT

1. Introduction

Nutrient cycling and energy flow are centered on photosynthesis and plant growth, for plant tissue forms the greater part of the earth's biomass. But all organisms participate, and their role is determined not simply by their biomass but by their catalytic reaction in different ecosystems. The major nutrient cycles are the carbon cycle, in which the organic energy cycle is implicit, the nitrogen cycle, the sulfur cycle, and the phosphorus cycle. The major energy cycles are the solar cycle and the hydrological cycle that is not only the source of the major part of living matter but also provides the medium for all organic cycles. The geometry of nutrient cycling is determined by the nature and distribution of sources and sinks, the most important being the atmosphere, the ocean and soil, and the availability of the major, minor, and trace elements, and other growth factors.

Cycling is a function of reaction rate, and turnover is determined by pool size and residence time. The greatest difficulty lies in the identification and measurement of cycling (Payne and Wiebe, 1978). The determination of isotopic ratios has provided a valuable guide to nutrient cycling, and fortunately stable and/or radioactive isotope species of all the major nutrient elements exist.

The role of protozoa in nutrient cycling and energy flow is determined by their bionomics. The distinctive features of protozoa are their small size, their high rate of reproduction, often through a complex life history, the high conversion efficiency of nutrients to new cell tissue, and their potentially high metabolic

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rates. They occupy a wide range of ecological niches, but their role is generally in association with a range of other microorganisms that function together as a microcosm. In such a system, the components tend to be soluble and insoluble nutrients, saprobionts assimilating the available nutrients, and micropredators accelerating turnover rates by constant grazing on the saprobiont populations.

In this chapter, nutrient dynamics in free-living microbial ecosystems, at both the cell and population levels, and the determination of turnover rates in communities of different population structure are discussed. The ecological constraints of the microhabitat limiting turnover rates and the structure and dynamics of the population will be examined in relation to nutrient cycling and energy flow.

2. Nutrient Dynamics

2.1. Nutrient Cycles and Energy Flow

The major factor affecting nutrient dynamics is whether a transformation is energy yielding or energy demanding. The two most important transformations are oxidation–reduction and the interchange between the organic and inorganic pools, the cell and the substrate. The energetics of the cell are centered in the hydrolysis and synthesis of ATP, and the major expenditure of energy and nutrients is in cell maintenance, including motility and feeding behavior, and cell synthesis and division (Fig. 1). On this conceptually simple scheme of microbial bioenergetics is based the mechanism of the major nutrient cycles. In the simplest case, that of a single cell, bioenergetics may be centered on the cell membrane, separating the organic from the inorganic cycles. In this case, nutrient cycling and energy flow can be related to simple parameters of cell size, cell mass, and the rate of metabolic reactions. The limitations of monaxenic culture may similarly be reduced to simple parameters. Where controversy exists is over the effects of food chains, particularly predation, on the rate of nutrient cycling. Opinions have fluctuated between those who consider that direct cycling by primary saprophytes provides the most rapid transformations and those who consider that a more complex food chain, involving prey–predator relations, accelerates mineralization and hence cycling. Because they constitute the most notable microbial predator in most natural microcosms, this controversy has centered particularly on the role of protozoa as micropredators.

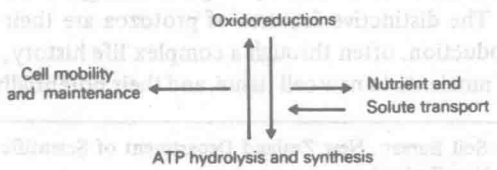

Figure 1. Bioenergetic relationships (after Garland, 1977).

Nutrition and microbial interaction involving protozoa have been recently reviewed (Curds, 1977), and in this section only the literature directly pertaining to nutrient and energy cycling is covered.

2.1.1. Single Populations

2.1.1a. Carbon. Carbon is central to nutrient cycling both because of its relation to energy and because, apart from water, it comprises the greatest bulk of protoplasm. Two aspects are important: the respiration or fermentation of carbon, and the relation of assimilation to carbon loss; and secondly, the intrinsic rate of natural increase. Unicellular organisms, and protozoa in particular, differ from metazoa, both heterotherms and homotherms, in their metabolic rate and their intrinsic growth. Whereas for a heterotherm of comparable size, the metabolic rate is 8.3 times greater than that of the protozoan, its intrinsic growth rate is only about twice as great. Thus, the more complex organism requires a greater proportion of assimilated energy for maintenance and consequently is less efficient as a secondary producer (Fenchel, 1974). Put more simply, the same nutrient and energy resources will sustain a much larger protozoan population than metazoan population, and similarly nutrients and energy cycled through a protozoan population will be conserved more efficiently than if cycled through a metazoan population The peculiar status of protozoa in nutrient cycling turns on this point. The second point of ecological importance is the dependence of the intrinsic growth rate on cell size, smaller protozoa having a shorter generation time than larger protozoa (Fenchel, 1968b; Finlay, 1977). The actual metabolism of carbon substrates by protozoa in most decomposition processes does not differ biochemically from that of other organisms, the same mechanisms and pathways operate as in many bacterial and animal cells (Stout, 1974), and as with other organisms, their metabolism is regulated by availability of substrate and environmental conditions. There are, however, intrinsic differences in metabolic rates between the different protozoan groups and between different species of the same group. Lee and Muller (1973) discussed metabolic activity of some salt-marsh foraminifera which have a relatively high rate of metabolism but show considerable variation between species. The testacea, on the other hand, appear generally to have low metabolic activity, though some species may be comparable to the foraminifera. Although the small amebae have quite a high rate of respiration, ciliates appear to have a much higher rate, although both are dependent upon an adequate food supply (Stout and Heal, 1967), and the cysts have a much lower respiratory rate than the trophic cells. Protozoa, of course, may also assimilate CO_2 in the fermentation of glucose to succinic acid (Peak and Peak, 1977; van Niel *et al.*, 1942), thus constituting a sink as well as a source of carbon flow.

2.1.1b. Nitrogen. Normally most protozoa assimilate amino acids and excrete ammonia. Some, such as *Chlamydomonas*, may be able to assimilate nitrate (Nichols and Syrett, 1978). When protein or an amino acid is used as an

energy source, the ammonia released is excreted, but starved cells may assimilate ammonia as endogenous carbon sources are metabolized (Harding, 1937a,b; Doyle and Harding, 1937).

2.1.1c. Phosphorus. Because of its importance in both the energy and nutrient cycles, the metabolism of phosphorus by protozoa is of key importance (Matheja and Degens, 1971). The first search for labile phosphorus in protozoa was by Needham *et al.* (1932), with negative results, but Kandatsu and Horiguchi (1962) showed the presence of ciliatine, 2-amino ethylphosphonic acid (with its C—P bond) in the ciliate *Tetrahymena pyriformis*. Now the role of the nucleophosphatide pools in both the ciliate *Tetrahymena* and the small ameba, *Acanthamoeba*, is well worked out. Study of changes in the ADP, ATP, and AMP levels of *Acanthamoeba* during different stages of development have shown differences in concentration between the logarithmic growth phase and the stationary phase, when encystation takes place (Jantzen, 1974; Gessat and Jantzen, 1974). Encystment is accompanied by a depletion of total adenosine phosphate to about 85% because of the depletion of ATP. During development, the energy charge becomes stabilized between 0.58 and 0.81, characteristic of the different modes of encystation. During this cell cycle, changes also occur in the cytoplasmic membranes and alkaline phosphatase activity (Pauls and Thompson, 1978), and in the carbon cycle, in the activity of cytochrome oxidase, dehydrogenase, and catalase, associated with changes in the nucleophosphatides (Edwards and Lloyd, 1977a). Such changes are also correlated with cyanide sensitivity of the respiration (Edwards and Lloyd, 1977b). Edwards and Lloyd (1978) also showed oscillations in pool levels of ATP, ADP, and AMP during the 7- to 8-hr cell cycle (Fig. 2). Roti and Stevens (1975) showed that encystment was associated with the breakdown and synthesis of DNA and that inhibition of encystment was correlated with inhibition of phosphate incorporation. Respiration rates, adenine nucleotide levels, and heat production were measured during exponential growth of *Tetrahymena pyriformis* by Lloyd *et al.* (1978), who found that the ATP pool oscillated in phase with respiratory activity. The ADP and AMP pools oscillated in phase with each other but out of phase with ATP. Values calculated for the energy charge were low (0.2–0.5). Echetebu and Plesner (1977) also followed the nucleotides during cell division (Fig. 3). *Tetrahymena* was grown in synchronized culture, and concentrations peaked 1 hr after the heat shock during the first division cycle and reached a minimum in the second cycle 2 hr after the heat shock. There was thus a complete turnover of adenine phosphates during this period. Similar results were reported by Lloyd *et al.* (1978), who followed O_2 uptake, heat production, and adenine nucleotide levels in exponential and synchronized cultures of *T. pyriformis*. They also found that the O_2 uptake and ATP pool oscillated in phase but not the ADP or AMP pools. Values calculated for adenylate charge were low, increasing from less than 0.2 to more than 0.4 in the mid-exponential phase of growth. There is thus a contrast in behavior of the adenylates between the ameba and the ciliate, and in both the

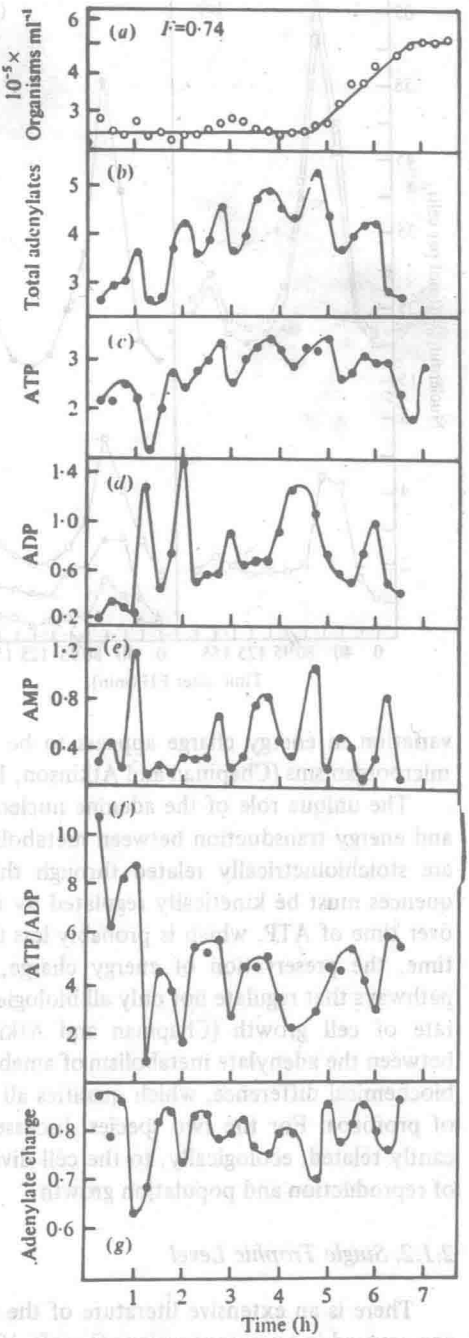

Figure 2. Energy metabolism in synchronous *Acanthamoeba*. Changes in adenine nucleotide pool levels and adenylate charge values in a synchronously dividing culture of *A. castellanii*. The synchronous culture contained 10% of the exponentially growing population; 1-ml samples were withdrawn at 15-min intervals for measurements of adenine nucleotides after rapid quenching and extraction in chloroform. (*a*) Cell numbers and synchrony index, *F*; (*b*) total adenylates; (*c*) ATP; (*d*) ADP; (*e*) AMP; (*f*) ATP/ADP ratio; (*g*) adenylate charge. Adenylate concentrations are expressed as nmol/ml culture. From Edwards and Lloyd (1978).

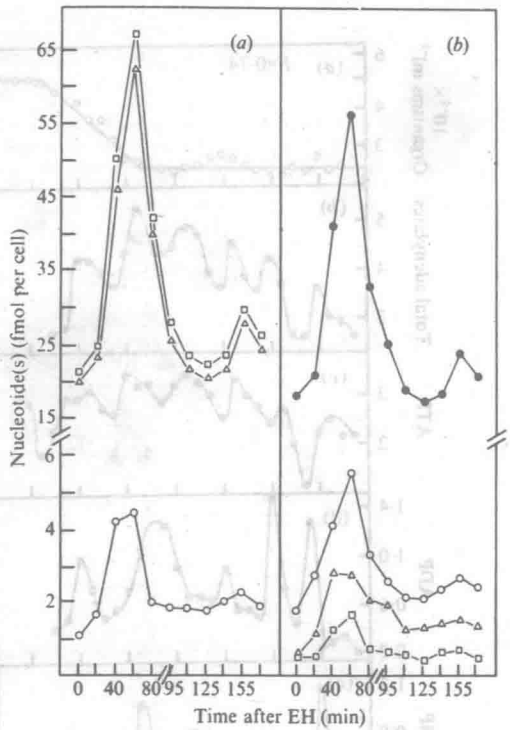

Figure 3. Amounts of ribonucleoside triphosphates in *Tetrahymena pyriformis* during the first and second synchronous division cycles. Cells were grown exponentially in PPY medium containing [^{32}P]orthophosphate (2–5 μCi mol^{-1}) for over six generations to achieve isotopic equilibrium, then induced for synchrony of divisions. At intervals after the end of the synchronizing heat treatment (EH), samples were taken, chromatographed, and the nucleotides were quantified. Results shown are the average from five experiments. (*a*) Sums of ribonucleoside triphosphates: □-, ATP + GTP + CTP + UTP; △, ATP + GTP; ○, UTP + CTP. (*b*) Individual ribonucleoside triphosphates: ●, ATP; △, UTP; ○, GTP; □, CTP. From Echetebu and Plesner (1977).

variation in energy charge appears to be at variance with previous reports for microorganisms (Chapman and Atkinson, 1977).

The unique role of the adenine nucleotides is in the stoichiometric coupling and energy transduction between metabolic sequences. All metabolic pathways are stoichiometrically related through this system, and similarly all such sequences must be kinetically regulated by it also. What is remarkable is the turnover time of ATP, which is probably less than 1 sec, and it is this short turnover time, the preservation of energy charge, and the coupling with biosynthetic pathways that regulate not only all biological homeostasis but also determine the rate of cell growth (Chapman and Atkinson, 1977). Differences, therefore, between the adenylate metabolism of amebae and ciliates point to a fundamental biochemical difference, which qualifies all generalities about the ecological role of protozoa. For the two species discussed, these differences are most significantly related, ecologically, to the cell division cycle and consequently the rate of reproduction and population growth.

2.1.2. Single Trophic Level

There is an extensive literature of the nutrition of axenic cultures of protozoa covered in numerous reviews (Lwoff, 1951; Hutner and Lwoff, 1955; Hutner,