

Technical Report No. 52  
THE MICROBIAL COMPONENT OF THE ECOSYSTEM<sup>1</sup>

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U. S. International Biological Program

June, 1970

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<sup>1</sup>Paper presented to the US-IBP Desert Biome Microbiological Specialists' Meeting held at Las Cruces, New Mexico, April 3-4, 1970.

The microbiologist who forsakes his isolationism and trespasses into ecosystem studies very quickly finds that he must talk to a whole array of botanists, mammalogists, entomologists, ornithologists, ecologists and climatologists--all of whom are firmly convinced that their own particular specialty is the absolute crux of the entire biome. As microbiologists we can easily believe that the soil microflora accounts for one-half the total dissipation of the net primary productivity, but most of us refuse to extend our natural gullability to the extent of believing that each of several other ecosystem components dissipate a similar fraction. Two hundred percent dissipation is staggering even for a microbiologist.

Not surprisingly, the figure of fifty percent dissipation by the ecosystem microflora is staggering to all those ecosystem workers who are not microbiologists. Recently I undertook to draw up from the literature a table to show that, excepting for the dominant primary producers, the microflora is not only the major component of the total biomass but also is the major furnace for burning up the energy materials stored annually by photosynthesis. I was confident that such a table, once constructed, could also be used to show the high rate of metabolic activity of the microflora. As one progressed down the size scale from large mammals to microbes, one would expect the ratio between the standing crop of a given component group and the annual flow of energy through that component to become progressively larger. Such a progression, from 10 to 600, is shown in Table 1 as one moves down the size scale from mammals to nematodes. For the microbes, one would expect the ratio to widen even further, perhaps to 1000. Instead, there is a precipitous narrowing, to a value even

lower than that given for elephants. Clearly, a discrepancy of this magnitude demands some attempt at explanation.

The first question to be asked is whether or not, with a microbial biomass of  $200 \text{ g/m}^2$ , an annual microbial energy flow of  $2,500 \text{ kcal/m}^2$  is a reasonable accomplishment for that amount of biomass. Oginsky and Umbreit (1955) have stated that the microbial maintenance energy requirement is  $50 \text{ kcal/day/g}$  of cell nitrogen. For  $200 \text{ g}$  of microflora/ $\text{m}^2$ , one can assume  $20 \text{ g}$  of cell nitrogen, hence  $1,000 \text{ kcal/m}^2/\text{day}$  would be needed for the microbial standing crop. Given the Matador primary productivity, that site should support its "measured" microflora for only 2.5 days each year. A similar discrepancy is encountered if one undertakes a calculation based on the energy requirement for microbial cell production. According to Camp (1963), each gram of dry cell weight synthesized requires the equivalent of  $1.4 \text{ g}$  of glucose. Therefore  $200 \text{ g}$  of microbial biomass would require  $280 \text{ g}$  of glucose, or roughly  $1200 \text{ kcal}$ , for each generation of microbial cells produced. Under this requirement, the soil microflora could reproduce its standing crop of biomass only a very few times, possibly only once or twice, each year.

Using either the cell nitrogen calculation or the cell production calculation and assuming 200 days of microbial growing season or 200 microbial generations per year, the yearly energy requirement for  $200 \text{ g/m}^2$  of microbial biomass would be of the order of  $200,000 \text{ kcal/m}^2$ . If one bears in mind that microbial cells in the logarithmic growth phase metabolize at rates ten or more times their maintenance energy level requirements, or bears in mind that 70 percent efficiency in converting glucose to cell substance is a high estimate--it can be of the order of only 10 percent--then one can visualize an impossibly high microbial annual energy requirement of the order of several million  $\text{kcal/m}^2$ .

One can assume that  $200 \text{ g/m}^2$  of microbial biomass is an erroneously high estimate and turn his attention to actual soil metabolism values that can be measured in the soil itself. In my own experience I have found that following addition of 1 g of glucose to 100 g of soil in the laboratory, 50 to 60 percent of the glucose carbon is recoverable as  $\text{CO}_2$  in the first three days. This shows a rate of glucose metabolism equivalent to  $300 \text{ g of glucose/day/m}^2$  to a depth of 15 cm, or a respiratory energy flow of  $1200 \text{ kcal/m}^2/\text{day}$ . This rate was achieved without any pre-baiting of the soil with glucose and without any special effort to provide the soil with additional aeration or with nitrogen, phosphorus, sulfur or other ammendments.

In brief, whether one starts with an actual biomass measurement and then makes calculations based on statements by Oginsky and Umbreit (1955) or by Camp (1963) or whether he uses an actual soil metabolism measurement, the results indicate an improbable order of magnitude of microbial energy flow of from several hundred thousand to a million or more  $\text{kcal/m}^2/\text{year}$ . However, should  $800 \text{ kcal/m}^2$  of standing microbial crop achieve such a potential energy flow, then the ratio between the standing crop and annual energy flow becomes of the order of 300 to 3000, at which level it would not be out of line with the other ratios given in Table 1.

The several above calculations indicate very clearly that the microbial biomass observable in soil largely represents a potential activity, a potential that is only in part realized. In soil, generally, the individual segments of the soil microflora must be active only sporadically. The concept that soil permits the survival of a great many microbial cells during periods in which they are not

Table 1. Biomass and annual energy flow values for various consumer and decomposer subgroups<sup>1/</sup>.

	I. Biomass, kcal/m <sup>2</sup> .	II. Energy flow, kcal/m <sup>2</sup> /yr.	Ratio, II/I.
Large mammals	2 to 20	to 200	10
African elephant	5.1	23.1	5
Uganda kob	3.1	62.4	30
Small mammals	0.1 to 2	to 200	50
Birds	.05	2.5	50
Enchytraeids	3.0	to 150	50
Leaf hoppers	1.0	300	300
Nematodes	0.5	300	600
Microflora	800	2,500	3 !

<sup>1/</sup> Values other than for microflora taken mostly from Petrusewicz (1967).

Microfloral values based on Matador grassland site (Canada) in which the microfloral biomass has been measured as 200 g/m<sup>2</sup> and the annual net primary productivity has been estimated at 5000 kcal/m<sup>2</sup> (Clark and Paul 1970).

metabolically active was expressed several years ago by Clark (1967). and more recently it has been reiterated by Babiuk and Paul (1970). The obvious implication of this concept is that microbial biomass and/or numbers cannot be viewed in the same perspective as can the biomass or census numbers of other consumer and decomposer organisms in the ecosystem. A given amount of elephant or of mouse biomass or a given population of elephants or of mice represent within reasonable limits of variation a predictable amount of annual energy flow. In contrast, a gram of microbial biomass may represent essentially non-measurable metabolism, as is the case in air-dried or lyophilized cultures of bacteria that remain viable on the laboratory shelf for periods up to 50 or 100 years, or it may represent a very active metabolism in which the microbial cells require several times their own weight of food material each day.

The above musings lead me to speak very unkindly about the use of plate of dilution counts of soil microorganisms in ecosystem studies. A preceding speaker on this program has remarked that many microbiologists are so loathe to update their thinking that they might well be categorized as belonging to the era of the dinosaur. Although he did not spell out his indictments, I am fairly confident that one of the points he had in mind was the slavish devotion of far too many microbiologists to census counts. Personally, I am perfectly willing to state that as a measure of microbial activity in soil, a total population count is not just worthless, but absolutely worthless. It is encouraging that in the past year or so the attendees at several microbiological symposia have stated summarily that making such counts is industry mis-directed.

Whether or not it is worthwhile to measure the total microbial biomass remains controversial. Biome workers are usually charged with determining the biomass of their particular structural component, possibly because biome directors are large mammal or mouse or grasshopper oriented, in which compartments biomass is much more meaningful than in the microbial compartment. At this conference some participants have espoused the value of total biomass measurements while others, for at least certain purposes, are willing to lump the soil microbial biomass into the same compartment as the soil organic matter. In the latter group I refer especially to Dutt (1960) and co-workers and to the nitrogen models that they have developed. For their purposes I quite agree that such a combination may be a reasonably valid simplification and perhaps quite necessary in order to achieve an operational model.

Nevertheless it must be kept in mind that such a simplification is for a specific purpose and I do not think it will be found equally suitable for process and energy flow studies in ecosystem microbiology generally. I am more hopeful that in lieu of total biomass measurements or total carbon measurements some of you will be successful in developing methods of measuring biomass activity. Worthy of mention here is the work of Doxtader (1969) on ATP measurements and Skujins' interest in dehydrogenase measurements. To understand the role of soil microorganisms in the ecosystem, one needs functional rather than morphological measurements. I sometimes think an ecosystem ecologist is a biologist who carves up his ignorance into a series of little black boxes and thereafter spends his time in drawing arrows between boxes. Can the microbiologist meet the challenges thrust at him and come up with information to be used in lieu of little black boxes?

How can one measure microbial activity in an ecosystem? Perhaps the **oldest** and most widely used method is respirometry. Procedures applicable to field conditions have been developed. The chief criticism of the method is that while the total respiration can be measured, there is no easy way to subdivide or apportion that total among such contributing components as the soil microflora, the live root biomass and the invertebrate fauna. If one uses selective elimination of one or more of such components, there is disturbance of the natural conditions that he is attempting to study.

The use of  $^{14}\text{C}$  to tag plant materials for use in litter decomposition studies not only permits recognition of the  $\text{CO}_2$  evolved from such litter but also permits identification of derived and residual organic materials that remain in the soil. Dahlman (1968) has used  $^{14}\text{C}$  to measure the rate of turnover of the root biomass of grasses in the field. Other workers have used carbon tracing and dating techniques to show that the organic components of soil can be subdivided into three dynamic fractions: (a) decomposing plant residues and the associated microbial biomass which turn over at least once every few years; (b) microbial metabolites and cell wall constituents that become stabilized in soil and possess a half life of 5 to 25 years; and (c) the resistant humic components ranging in age from several hundred to several thousand years (Clark and Paul, 1970).

Much can be done without elegant instrumentation. In his report to this meeting describing a respirometer for forest litter studies, Gilmour (1970) has indicated some success in the development of a quick extraction and oxidation procedure that measures a readily decomposable fraction in the litter. The values obtained showed good correlation with respirometry data obtained during 28 days of incubation. In my own recent work I have been using a very simple procedure involving cellulose



filter paper burials and retrievals. Thus far the technique has been found quite informative and quite sensitive to differing soil conditions both in the field and in the laboratory. It has shown the slowing down of field microbial activity in the late fall, almost complete cessation of activity during the winter, and slow resumption of activity during the early spring. Table II shows some representative data for differing periods of incubation following addition of supplemental nutrients in the laboratory.

The response to added nutrients was not unexpected in the laboratory wherein nearly optimal moisture and temperature conditions could be provided. It was somewhat surprising to find that added nutrients tripled the decomposition rate of field buried cellulose during the winter months of 1969-1970. During the period the soil was frozen much of the time, and the highest soil temperature observed for the soil depth at which the cellulose paper was buried was 5° C. The work to date has raised such additional questions as: What is the effect of added nutrients on the decomposition of naturally occurring field litter materials during either the cold season or during a warm, moist season? Does the plant chemical composition of different plant species, or that of a single species at differing stages of its maturity, play an important role in determining the rates of decomposition of the individual litters? What is the effect of surface placement versus soil burial on litter decomposition? Of different depths of soil burial? What is the role of the soil invertebrate fauna in litter decomposition?

At the outset of this discussion I dwelt at some length on the thesis that the total microbial biomass in soil must be viewed as largely dormant at any given time, with the individual component subgroups springing into activity only when conditions were specifically favorable for them. In this

Table 11. Decomposition of cellulose in laboratory soil.

	14 days	28 days	42 days
	<u>Percent recovery of added cellulose</u>		
Untreated	73.8	47.5	11.3
plus P,K,S <sup>1/</sup>	74.4	49.2	14.4
plus N	44.9	30.8	6.1
plus N, PKS	25.6	19.0	5.4

<sup>1/</sup> Nutrient additions were made on the basis of filter paper carbon and according to the following C:N:P:K:S ratios: 100:10:1:1:1.

context the soil microflora is a consortium of chemical specialists and it is not only the nature of the substrate but the conditions under which that substrate is presented that determine which microbes dominate substrate transformation (Clark 1965). With respect to ecosystem energy flow and nutrient cyclings, this simply means that the decomposition of any given substrate may be accomplished by one organism under some conditions and by other organisms under other conditions. One must be cautious about assigning too much importance to any single type or group of decomposer organisms on the basis of limited observations.

With the advent of the nylon net litter bag it became possible to expose given quantities of litter under field conditions and to measure rates of disappearance of the litter. By varying the size of the mesh openings or else by treating the litter with repellants, in order to exclude or not to exclude members of the soil fauna, it appeared possible to measure the importance of the invertebrate fauna in the processes of decomposition.

Some rather striking data were published. Edwards and Heath (1963) reported that litter exposed in bags to which earthworms had access decomposed three times faster than when the earthworms were excluded. Crossley and Witkamp (1964) found that 55 percent by weight of oak leaf litter was retained after one year in plots treated with naphthalene versus 40 percent retention in control plots. Kurcheva (1960) in similar work found that naphthalene-treated oak litter lost only 9 percent of its original weight in 140 days whereas untreated litter lost 55 percent in the same time. Perhaps largely on the basis of such experiments, writers of soil fauna papers have ascribed rather large annual energy flows to the soil invertebrates (Macfadyan 1963; Paris 1969).

A number of recent workers have come to quite different conclusions. Some have suggested that much of the weight loss from nylon-bagged litter represents not decomposition as such but animal transport of litter from the bags as well as acceleration of gravitational litter losses because of animal movements in the litter. Curry (1969) in a study of the role of soil animals in the decay and disappearance of herbage from nylon bags of different mesh sizes found that animal activity contributed little to the rate of decay and disappearance of herbage on the soil surface and did not accelerate the decay of herbage in the soil. Malone and Reichle (1970) found that litter confined in mesh bags disappeared most rapidly in pens treated to eliminate all invertebrate fauna. Neither the earthworms nor arthropods appeared significant in the breakdown of litter--indeed, it appeared that the invertebrate fauna may disturb the microflora and interfere with their optimum potential in decomposing litter. Will (1968) in a study of coniferous litter found that although litter in bags differed greatly in appearance from that to which the larger invertebrate fauna had access, the chemical composition of the two litters was essentially similar.

Macfadyan (1963), in the paper which has been so widely cited by later workers to emphasize the role of the invertebrate fauna in decomposition, stated very clearly that his estimations of energy flow were very approximate indeed. He noted that data based on gas analyses indicated a respiratory rate about 50 times as great as the total rate of supply of organic matter to the soil. His statement that "direct measurements on soil microorganisms referable to conditions in nature do not seem to have been made" is especially worthy of emphasis. The biome microbiologist who visits the field only once or twice a year and then only for the purpose of transporting a teaspoonful of soil to the laboratory for intensive study under

laboratory conditions may accumulate new and valuable information, but until he makes his measurements referable to conditions in the field, he will fail to contribute information useful to his co-workers who are interested in the flow of energy and the cycling of nutrients in the ecosystem.

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