

NUTRIENT AND ENERGY FLOWS THROUGH SOIL MICROBIAL BIOMASS

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INTRODUCTION

The recognition and recent studies of the role of living organisms (biomass) in carbon, nitrogen and phosphorus flows in nature have stressed the role of the microorganisms as sources and sinks for nutrients, in addition to their traditional role in transformation of the nutrient elements. Techniques for determining the size and activity of laboratory cultures have had to be altered for field measurements. Similarly, growth concepts developed using liquid culture studies should be applicable in the field after the effects of such factors as competition, the presence of inhibitors, physical adsorption and entrapment, and the broad variety of available energy sources have been considered.

Enough information is now available on microbial biomass and activity in terrestrial soils, sediments and aquatic systems that reasonably accurate mathematical descriptions of the pool sizes and processes involved can be made. Mathematical modelling is useful in that it allows one to describe the processes. It also provides for the testing of available data and concepts and thereby points out where further research is necessary.

MICROBIAL GROWTH AND CARBON TRANSFORMATION MODELS

One example of the role that microbial biomass and activity play in carbon cycling can be found in the description in the decomposition and stabilization of organic matter in grassland soils (Fig. 1). In compiling the information necessary to obtain a meaningful mathematical expression for the biological, chemical and physical processes involved in organic matter turnover under both native and cultivated conditions, it was found that reasonably good data are available for plant

decomposition rates. Net decomposition rates in the laboratory, under optimum moisture and temperature conditions (van Veen and Paul, 1980), were found to be twice those in tropical soils of Nigeria (Jenkinson and Ayanaba, 1977). In turn the decomposition rates in the moist temperature climates of Europe were a quarter of those found in Nigeria (Jenkinson and Rayner, 1977). A Canadian grassland with a dry summer and cold winter had an average annual decomposition rate one-twentieth of that found in the laboratory under optimum conditions. The rates during the one or two warm moist months were higher than those found in Europe on soils.

Determination of plant decomposition rates requires a knowledge of not only net rates, that is, the amount of ¹⁴C-carbon left behind but also involves correction for microbial growth and production of soil organic matter so that gross rates can be determined. Decomposition rates corrected for microbial utilisation of the substrate at reasonably high efficiencies (35 to 60%) show much faster transformation rates (Table 1) than usually described in the literature, and most field studies have not utilised short enough time intervals to obtain the adequate measurement of the kinetics involved during the early decomposition periods.

TABLE 1
Organic matter levels, annual input of plant residues and optimal decomposition rates for a grassland soil (Sceptre)

Material	Amount present (kg C ha ⁻¹ 80 cm ⁻¹)	Decomposition rate constants (day ⁻¹)
Litter	1,000	8 x 10 ⁻²
Root	10,300	8 x 10 ⁻²
Biomass	1,250	3 x 10 ⁻²
Decomposable organic matter not protected	4,400	8 x 10 ⁻²
Decomposable organic matter protected	44,100	8 x 10 ⁻⁴
Recalcitrant organic matter not protected	17,000	8 x 10 ⁻⁶
Recalcitrant organic matter protected	17,000	8 x 10 ⁻⁸

The description of microbial growth and soil organic matter formation (Fig. 1) has divided the plant residues into two fractions; a decomposable fraction and one that is recalcitrant. This agrees with present day concepts of humus formation which indicate that recalcitrant materials, such as lignins and polyphenols, enter the decomposable native soil organic matter fraction. Physical-chemical condensation with nitrogen rich products of microbial growth results in the formation of resistant humic materials (Haider, Martin and Filip, 1975).

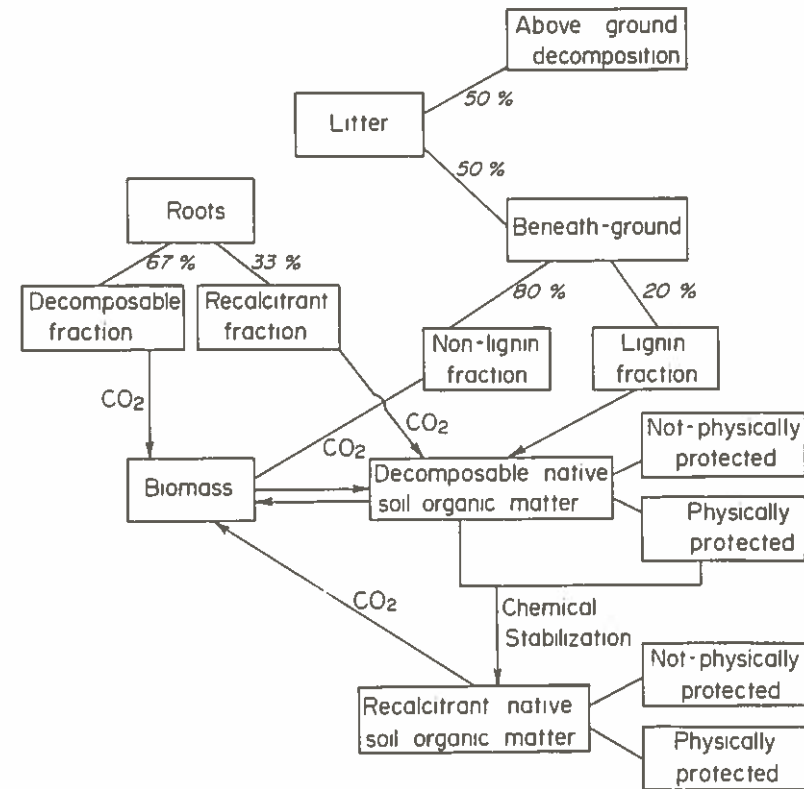


Fig. 1. Scheme of the long-term carbon turnover model.

In this study the modelling procedure was simplified by using primarily first order kinetics rather than the more complex hyperbolic equations involving microbial growth (Michaelis-Menten or Monod equations). This proved feasible because the soil biomass is so large (2% of the total carbon and 4% of the soil nitrogen) and has such great growth potential relative to the rather low amount of available substrate that one long term basis, such as the 400 years with subdivisions of 0.1 year used in this model, its initial size seldom limited microbial transformations.

Stabilized organic materials persist for long periods of time in sediments as well as in terrestrial soils. Carbon dating has indicated that the 40-60% of the carbon of terrestrial soils which is non-acid hydrolyzable has persisted for at least 1000 years and thus has very low decomposition rates. In natural systems, nearly all the reactions occur on or in solid matrices. This includes colloids consisting of humic materials, sesquioxides and fine clays. Amino acids added to microbial cultures or to soil have high turnover rates (Schmidt, Putnam and Paul, 1960; Marumoto, Kai, Yoshida and Harada, 1977).

The decay rate of amino acids in solution culture relative to those produced and stabilized within the soil was used to develop a soil physical protection factor (van Veen and Paul, 1980). Although the added acetate had disappeared from the system within four days (data not shown), the total ^{14}C -carbon remaining in the soil was stabilized at approximately 25% (Fig. 2). Microscopic examination indicated two populations of microorganisms had developed sequentially during the one to ten day incubation period before stabilization had taken place (McGill, Paul, Shields and Lowe, 1973). There are many conditions especially when glucose is added to moist field soils where stabilization occurs at much higher levels (Ladd and Paul, 1973; Paul and McLaren, 1975; Wagner, 1975).

During computer simulation of the decay of the added ^{14}C -acetate and of the transformations of the microbially produced ^{14}C -amino acids, the best fit of the data in Fig. 2 was obtained by assuming 50-60% of the amino acids were protected. Non-protected amino acids had a degradation rate of 0.3 day^{-1} . Protection changed this by a factor ranging from 0.01 to 0.005 times the unprotected rate. It was found that the degree of protection, not the initial decomposition rate, controlled the amino acids present in the long term incubation (van Veen and Paul, 1980). The 50-60% protection of organic materials agrees with published data of the amount of organic materials closely associated with soil solids (Legg, Chichester, Stanford and De Mar, 1971; Anderson, 1979).

Physical protection controlled the decomposition rate of both the decomposable and resistant compounds in the model

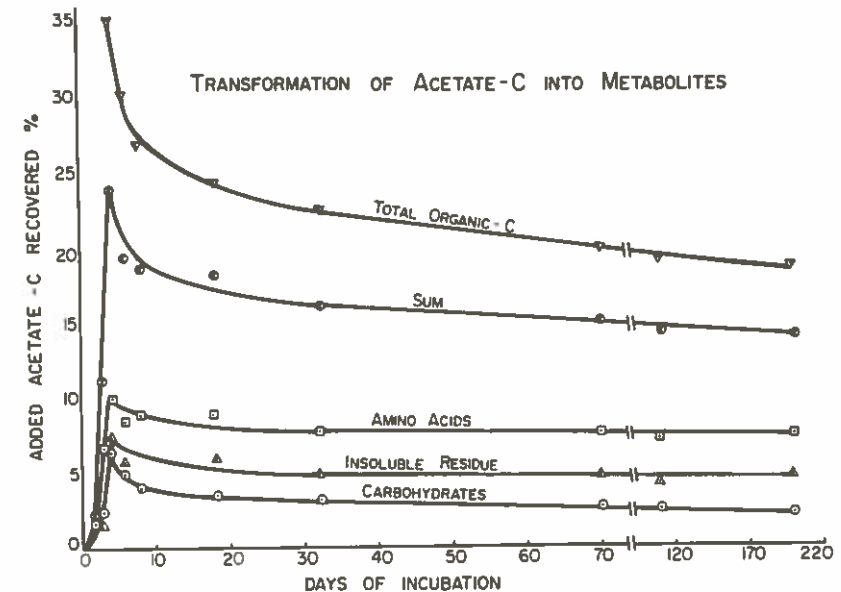


Fig. 2. Recovery of labelled carbon from soil during decomposition of ^{14}C -labelled acetate (data from Sørensen and Paul, 1961).

shown in Fig. 1. This differs from the model of Jenkinson and Rayner (1977) in which physical protection affected only the readily decomposable compounds. Materials such as clay-adsorbed amino compounds although degraded at much lower rates than free amino acids still persist in the soil for much shorter periods of time than humates which also may or may not be physically protected.

The alteration of physical protection by cultivation is one of the major factors controlling the availability of substrate to terrestrial microorganisms. More data on the interactions of organic materials with solid surfaces are required.

MICROBIAL GROWTH AND NITROGEN AND PHOSPHORUS CYCLING

The transformations of organic nitrogen are closely related to those of carbon. Processes such as dinitrogen fixation and denitrification are driven largely by the energy derived from the oxidation of carbonaceous materials. The C:N ratio of microorganisms can range widely but in most cases it ranges between 5 and 15. Mineralization-immobilization rates of

nitrogen can be calculated once an understanding of microbial growth relative to carbon cycling is available (Paul and Juma, 1980).

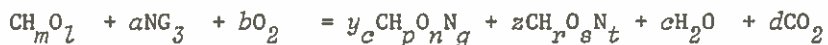
Phosphorus transformations are not as closely related to carbon flows as are those involving nitrogen. However, phosphorus cycling studies indicate a high interrelationship between inorganic phosphorus availability and microbial activity (Hannapel Fuller and Fox, 1964). Simulation models of phosphorus cycling indicated that annual flow through the microbial biomass was greater than that through above and beneath ground plant phosphorus. The major controls in these models (Fig. 3) were inorganic phosphorus solubility, soil water content and rates of diffusion of phosphorus in soil (Stewart, Halm and Cole, 1973; Cole, Innis and Stewart, 1977). Microbial transformations of labile and stable organic phosphorus often produce the majority of the plant available solution phosphorus.

The principle pathways in the cycling of phosphorus within the detritus and sediment of aquatic systems (Parsons *et al.*, 1977, quoted by Fletcher, 1979) contained pools similar to those in soils. Zooplankton have long been considered to be a major factor in the mineralization of organic phosphorus in aquatic systems. Growth studies with mixtures of pseudomonads, amoeba and nematodes (Cole, Elliott, Hunt and Coleman, 1978; Coleman, Anderson, Cole, Elliott, Woods and Campion, 1978) have confirmed earlier suggestions (Cutler and Crump, 1935) that the soil fauna can play a major role in soil biomass dynamics and subsequently in the mineralization of nutrients such as organic phosphorus.

MICROBIAL GROWTH, BIOMASS AND PRODUCT FORMATION IN THE SOIL

The close interactions of microorganisms with soil solids and the low apparent growth rates of a very large soil population have meant that interpretation of the effects of microbial growth in terrestrial systems has lagged behind studies using chemostats and those in aquatic systems. Descriptions of microbial growth in sewage sludge, rumen and in single cell protein formation (Stewart, 1975; Harrison, 1978; Nagai, 1979) have added information that with proper interpretation should help in understanding the transformations in the solid-colloidal systems of soils and sediments.

The energy of organic substrates may be incorporated into biomass, evolved as heat, or incorporated into extracellular products as shown in the following equation (Erickson, 1979):



CH_mO_l denotes the elemental composition of the organic substrate, $\text{CH}_p\text{O}_n\text{N}_g$ is the elemental composition of the biomass, and

$\text{CH}_r\text{O}_s\text{N}_t$ is the elemental composition of any extracellular products.

y_c is the fraction of utilized organic substrate carbon converted to biomass,

z is the fraction converted to extracellular products, and d is the fraction converted to carbon dioxide.

PHOSPHORUS CYCLE

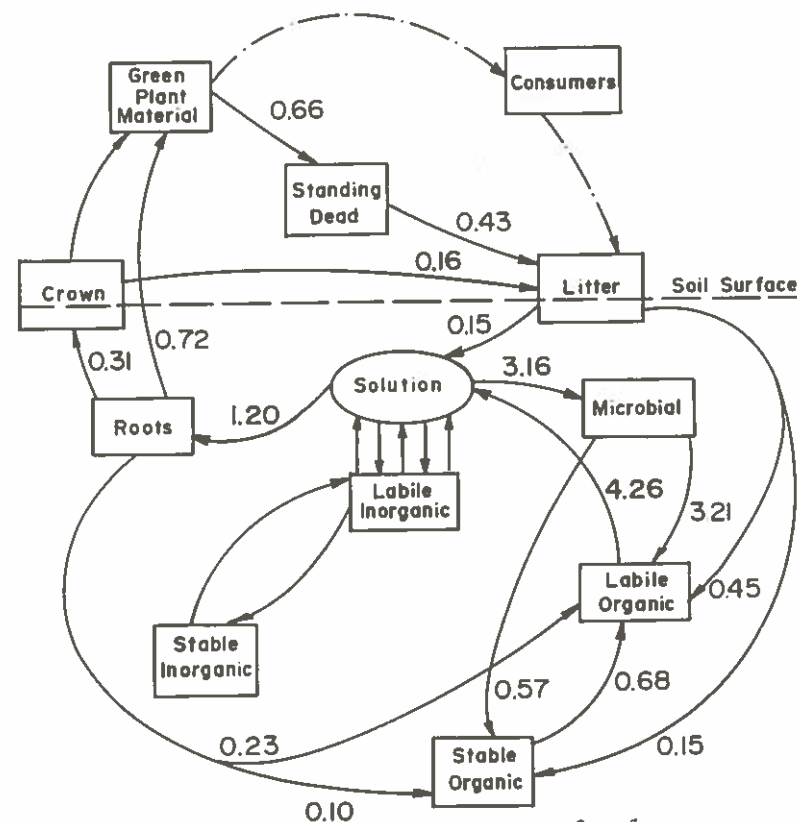


Fig. 3. Predicted flow of phosphorus ($\text{g m}^{-2} \text{y}^{-1}$) between components of a native grassland ecosystem (drawn from Cole *et al.*, 1977).

The overall energy of consumption can also be divided into that utilized for growth and that consumed for maintenance of population (Pirt, 1965).

Substrate consumption = Rate of consumption for growth + Rate of consumption for maintenance

From this : $\frac{\mu x}{Y_e} = \frac{\mu x}{Y_{eg}} + mx$

where μ = specific growth rate,
 x = organism concentration,
 Y_e = yield of organisms,
 Y_{eg} = theoretical yield, and
 mx = maintenance energy.

Harrison (1978) in discussing the efficiency of microbial growth recognized that resting cells require little energy to maintain cell integrity. He suggested that maintenance energy may represent one or a number of reactions:

- i) protection of cell integrity during active growth,
- ii) a constant leakage or wastage of energies from active cells due to inherent inefficiencies,
- iii) energy to maintain certain concentration gradients across the cell membrane, and
- iv) a constant death rate of the organisms.

The above equations describing microbial growth, maintenance energy and production formation were derived largely from chemostat studies. They have however been used by a number of authors to ask questions concerning the state and activity of soil biomass.

The maintenance energy value of 0.001 h^{-1} suggested by Babiuk and Paul (1970) was not a miscalculation of the value of 0.04 h^{-1} obtained by Pirt (1965) as suggested by Lynch (1979). The use of the low value by Babiuk and Paul (1970) and Gray and Williams (1971) was based on the recognition that maintenance energies determined in liquid cultures during active growth could not apply to a total soil population.

Parkinson, Domsch and Anderson (1978) also using the value of 0.001 h^{-1} concluded that with maintenance values of this magnitude approximately half of the carbon entering the soil would probably be utilized for maintenance, the rest would be available for growth of their measured population. Shields, Lowe, Paul and Parkinson (1973) calculated a maintenance value of 0.002 h^{-1} realizing that this value must also include cryptic growth and Behara and Wagner (1974) suggested a maintenance value of 0.003 h^{-1} . These estimates for the total soil population took into account the large proportion of live but relatively inactive cells which in the past could not be separated from the growing cells.

Early authors considered the high values for biomass found by direct microscopy to include a large proportion of dead cells. Recent work with ATP and the chloroform incubation techniques indicates that even higher values for the soil biomass. Therefore, it does not take a great deal of calculation to show that the population of natural systems although alive

and resistant to decomposition is largely dormant with only small proportions of the population actively growing or even having maintenance energy rates comparable to those found in the laboratory. Where doubling times of soil organisms have been calculated, these have usually been estimated to range from 40 to 50 hours (Coleman *et al.*, 1978; Domsch, Beck, Anderson, Söderström, Parkinson and Trollidner, 1979).

Van Veen and Paul (1980) in their modelling of microbial growth and soil organic matter formation stated that the concept of maintenance energy has been most useful to ecosystem microbiologists in asking pertinent questions concerning the size and activity of organisms in nature. However, not enough is yet known about these factors and cryptic growth in natural systems, therefore, in their mathematical analysis they incorporated the maintenance requirement of the population into the decay constant of soil organisms. This eliminated the need for separate maintenance energy constants for the various segments of the populations in various stages of activity.

THE SOIL BIOMASS

The biovolume in natural systems has classically been measured by direct microscopy. Visible light microscopy using cells stained with phenolic aniline blue (Jenkinson, Powlson and Wedderburn, 1976) or phase objectives (Parkinson, Gray and Williams, 1971) works well with the larger sized fungi. Fluorescence, particularly epifluorescence, makes it easier to see the smaller bacteria, and eliminates much of the interfacing background found in soils. Acridine orange has been utilized almost exclusively in aquatic studies. In soils, acridine orange, fluorescein isothiocyanate, magnesium sulfonic acid and europium chelate combined with a fluorescent darkeners have been utilized (Jenkinson and Ladd, 1980).

Attempts to measure that proportion of the population which is active have included examination with the phase microscope. The use of nalidixic acid to prevent DNA formation, followed by staining with acridine orange (Kogure, Simidu and Toge, 1979) and the epifluorescent detection of INT formazan in respiring cells (Zimmermann, Hurriaga and Becker-Birck, 1978) have been some of the attempts to measure active segments of soil and sediment populations. The possibilities that fluorescein diacetate (FDA) which does not fluoresce can be taken up by bacterial cells where it is hydrolyzed to the fluorescent fluorescein was tested by Babiuk and Paul (1970). They found that the uptake of the ester was too small in other than very young cells to be an effective staining technique. More recently, Söderström (1979) suggested this as a useable technique for measurement of the viable portions of fungi. However, in a

comparison of methods for soil microbial populations (Domsch *et al.*, 1979) found FDA to operate with a very low recovery of fungi.

The measurement of specific cell constituents such as lipo-polysaccharides (Watson, Novitsky, Quinby and Valois, 1977), mumaric acid (Miller and Casida, 1970; King and White, 1977; Moriarty, 1977) and fungal glucosamines is limited by the fact that these constituents occur in varying concentrations in different portions of the population; they can also occur as microbial products stabilized in soil organic matter. However, these biochemical techniques could have specific uses in microcosm studies and when using tracers so that radioactive constituents produced during microbial growth can be isolated from those generally occurring in soil organic matter.

The biovolume and not just the number of organisms must be obtained in direct microscopy (Jenkinson and Ladd, 1980). The conversion of biovolume to biomass has usually been carried out using values that assume 80% moisture by weight and 1.1 wet weight specific gravity. This resulted in a dry weight specific gravity conversion factor of 0.22 or a dry weight carbon content of approximately 0.11. Growth of soil isolates at moisture stresses often found in a soil led van Veen and Paul (1979) to suggest that the biovolume conversion rates for fungi should be 1.44 times the usual quoted figures of 0.22. They found very high densities for the bacterial isolates studied, but their fungal data are in agreement with values utilized by Parkinson *et al.*, (1978) and with density gradient measurements of actual soil organisms (Faegri, Torsvik and Goksbyr, 1977).

A number of authors have assumed both a high wet weight specific gravity and a fairly high moisture content. According to the calculations of van Veen and Paul, this is an anomaly as a microorganism of 1.3 to 1.5 specific gravity must contain less than the commonly assumed 80% moisture by weight. At this moisture level, the volume of water within the cell would be greater than the total volume of the cell originally utilized for the specific gravity calculation.

The cell constituent, ATP, has recently been measured with high recovery of internal standards and has shown high correlations with other techniques (Paul and Johnson, 1978; Bullied, 1978; Jenkinson, Davidson and Powlson, 1979). Studies with laboratory cultures (Lee, Harris, Williams, Armstrong and Syers, 1971 a,b) and aquatic organisms (Holm-Hansen and Booth, 1966) have indicated a general carbon to ATP ratio of 250:1 with the content of ATP being much higher in actively growing cells than in dormant cells. Jenkinson and Oades (1979) and Jenkinson *et al.*, (1979) compared the ATP content of the soil biomass with the carbon measured by the CHCl_3 -lysis incubation technique

and found much lower C-carbon:ATP ratios.

Jenkinson and his co-workers (Jenkinson, 1966, 1976; Jenkinson and Powlson, 1976) noted that ^{14}C -labelled microorganisms added to soil and lysed by agents such as CHCl_3 resulted in the evolution of fairly constant amounts of ^{14}C -carbon during subsequent incubation. Anderson and Domsch (1978a) found that labelled fungi lysed by CHCl_3 vapors were decomposed by a subsequent soil population and that 43% of the labelled CO_2 was evolved in 10 days; lysis of bacteria and subsequent incubation resulted in the evolution of 33% of the ^{14}C -carbon. Using a ratio of 1 to 3 for the distribution of bacterial and fungal carbon in the total soil biomass (Parkinson, 1973; Shields *et al.*, 1973), these workers calculated an average mineralization of the soil microbial population to be 41.1%. Thus the factor used to convert CO_2 -C evolved to biomass was:

$$\text{Biomass C} = \frac{\text{CO}_2\text{-C evolved}}{K_c}$$

where $K_c = 0.41$.

The extent of decomposition of low levels of radioactive substrate has been effectively utilized in aquatic systems to indicate microbial activity (Wright and Hobbie, 1966). This has been found to be difficult to interpret for soils and sediments. However, Anderson and Domsch (1978b) utilizing saturating levels of glucose have developed a rapid respiration technique for total microorganisms and have suggested delineation of the fungi and bacteria by utilizing antibiotics.

Comparisons of biomass data from CHCl_3 fumigation, direct counts and ATP measurements have been made for a number of soils. The values for soil biomass summarized by Jenkinson and Ladd (1980) ranged from 200 to 3500 μg carbon g^{-1} soil (Table 2). There was a good correlation between all three techniques. The major exception being that the acid deciduous wood of England gave very low results for the CHCl_3 fumigation technique. The laboratory incubation studies differed from the field studies in that the microbial populations were measured at a time of flux after the addition of substrate to soils with large variations in available phosphorus. In the latter study, the direct counts of fungi and bacteria have been adjusted to take into account the higher specific gravities of soil organisms (van Veen and Paul, 1979) and to include the organisms other than fungi and bacteria said by Jenkinson *et al.*, (1976) to constitute 36% of the soil biomass. This technique, however, still tended to give lower results than the fumigation measurements. The ATP content was sensitive to phosphorus availability and thus showed greater variations.

TABLE 2

Estimates of soil microbial biomass by different methods
(μg biomass carbon g^{-1} soil)

Soil	Chloroform fumigation	Direct counts	ATP content
Field soil data (referenced in Jenkinson & Ladd, 1980)			
Continuous wheat + manure, England	560	500	430
Continuous wheat, no manure, England	220	170	170
Calcareous deciduous wood, England	1230	1400	1040
Old grassland, England	3710	2910	-
Acid deciduous, England	50	300	470
Secondary rain forest, Nigeria	540	390	-
Arable cropping, Nigeria	280	240	-
Laboratory incubation (referenced in Paul & van Veen, 1978)			
Parent material, low P	330	200	400
Parent material, high P	180	170	512
Parent material with clay, low P	250	190	175
Parent material with clay, high P	190	190	237

Oades and Jenkinson (1979) and Jenkinson *et al.*, (1979) in studies of a heterogeneous group of soils, found a linear relationship ($r = 0.98$) between ATP content and biomass carbon content as measured by CHCl_3 -lysis-incubation. Domsch *et al.*, (1979) compared 15 different techniques used in assessing soil microbial biomass and activity. They also found that detailed quantitative data can be obtained by direct microscopy, the selective inhibition-incubation technique and ATP measurements. Data from enzyme assays were, however, difficult to transform to microbial biomass.

THE USE OF MICROBIAL BIOMASS STUDIES TO INTERPRET CARBON AND NITROGEN TRANSFORMATIONS IN SOIL SYSTEMS

The recently developed techniques for measurement of the soil biomass have made it possible to relate microbial growth and energy flow in a number of systems. Earlier calculations (Clark and Paul, 1970; Gray and Williams, 1971; Shields *et al.*, 1973) used the available data to obtain some indication of the

biomass size and turnover rate relative to energy supplies. More recent measurements have led to calculations of energy release and turnover rates within the rhizosphere (Barber and Lynch, 1977) in straw decomposition and during soil cultivation (Harper and Lynch, 1979; Lynch and Panting, 1980), and in a number of forest soils (Parkinson *et al.*, 1978). When Jenkinson (1966) used the fumigation technique to trace the decomposition of labelled ryegrass residues, he found that 31 to 39% of the labelled carbon remaining in the soil after one year's incubation was present as microbial biomass; 19% was present as cells after 4 years incubation. Shields, Paul and Lowe (1974) added ^{14}C -glucose in the field. Their fumigation studies showed that 75% of the ^{14}C -carbon remaining in the soil after 90 days field incubation was present as soil biomass. The rest of the labelled carbon was assumed to be present as extracellular metabolites or lytic products stabilized in the soil system.

Recent incubation studies in our laboratory utilized the above techniques, tracers and mathematical analyses to determine the movement of added substrate carbon and mineral nitrogen to biomass and product carbon and nitrogen. Measurement of the biomass at various stages during the incubation of soil with ^{14}C -glucose requires reincubation of the samples for 10 days following fumigation. The period of incubation after fumigation and the decision as to what CO_2 evolution values to use as a control have been major problems in the interpretation of this technique. Jenkinson (1966) assumed that the CO_2 evolved from the non-fumigated sample should be subtracted from the CO_2 evolved from the chloroformed sample during a 10 day incubation after fumigation. Jenkinson and Powlson (1976) and Anderson and Domsch (1978b) recognizing that the control soil can evolve large amounts of CO_2 during the initial stages of incubation suggested that the subtraction of CO_2 evolved during the 10 to 20 day incubation period.

The data in Fig. 4 show two methods of calculating both ^{14}C - and ^{12}C -carbon biomass during incubation with 1600 μg ^{14}C -glucose g^{-1} soil. Subtraction of the CO_2 evolved from the non-chloroformed soil proved difficult because the unfumigated soils had very high CO_2 levels. If the CO_2 content from the control was subtracted from the fumigated sample, the total soil biomass was found to rise from 425 μg carbon g^{-1} to 950 μg biomass carbon, with the non-labelled and labelled biomass present in approximately equal amounts (Fig. 4A).

Analysis of the glucose remaining in the soil (Fig. 5) showed that at the end of day 1, there was no glucose remaining in the soil although there is still a total of 950 μg ^{14}C -carbon g^{-1} . Figure 4A shows 500 μg of biomass ^{14}C -carbon at this time. One must conclude that the ^{14}C -carbon present was

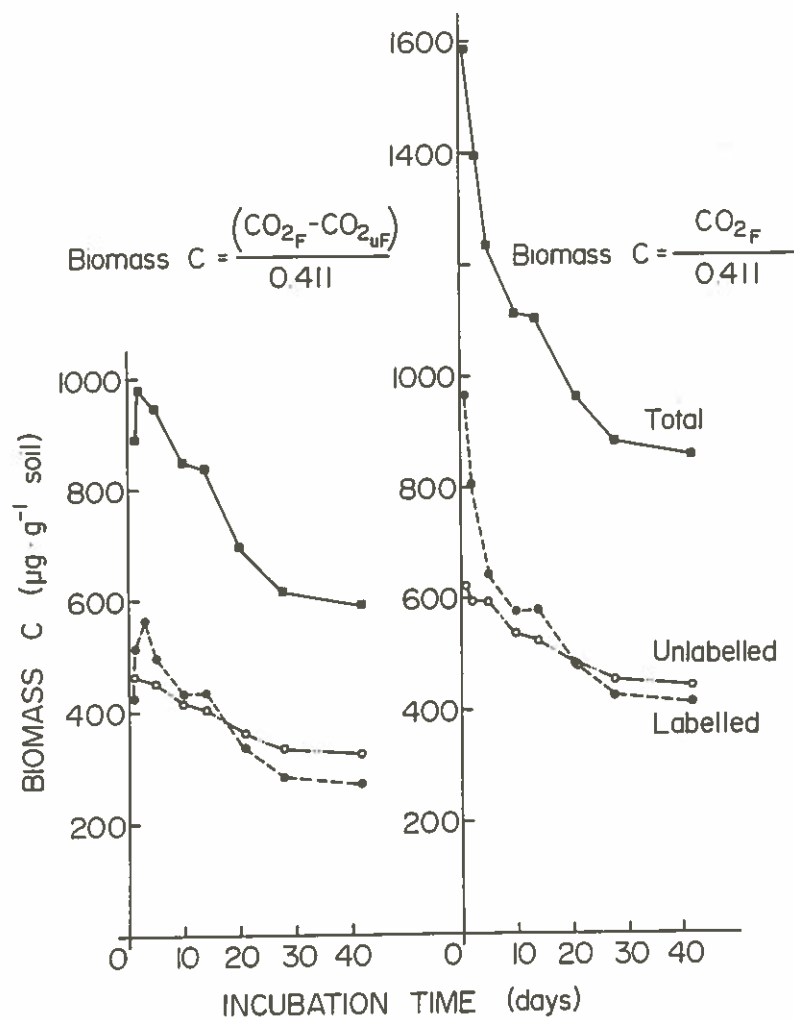


Fig. 4. Soil biomass determined with the CHCl_3 lysis incubation technique with (A) and without (B) subtraction of unfumigated soil CO_2 levels.

equally divided between the ^{14}C -biomass and ^{14}C -microbial products. It is difficult to envision such a large production of secondary metabolites or the occurrence of so many dead ^{14}C -cells immediately after the cessation of growth.

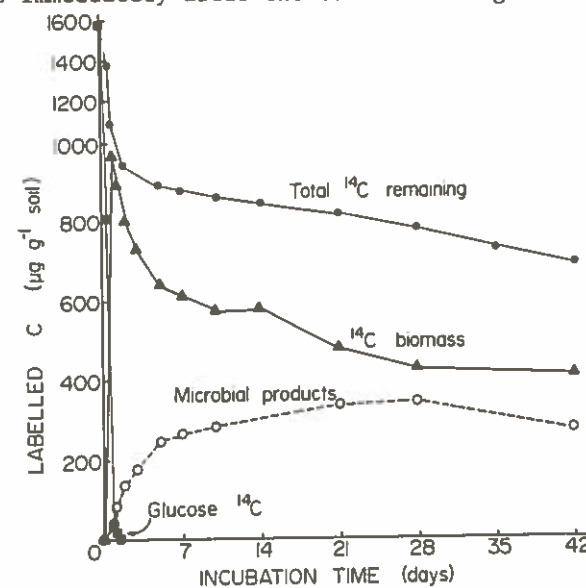


Fig. 5. Disposition of ^{14}C -carbon in soil.

Domsch and Anderson and Jenkinson and his co-workers in the development of the K factor for the CHCl_3 -incubation technique added ^{14}C -labelled microorganisms. These were subsequently fumigated and the CO_2 evolution measured. Their calculations therefore did not involve a subtraction of control ^{14}C - CO_2 levels.

In our study, all the original ^{14}C -glucose had disappeared by day 1; the remaining ^{14}C -carbon ($950 \mu\text{g carbon g}^{-1}$) should be in microbial cells. Fumigation of these samples and incubation for 10 days should result in a K_c value for *in situ* microorganisms. A total of $397 \mu\text{g } ^{14}\text{C-CO}_2$ were evolved during the 10 day incubation after fumigation. The K value when no control was subtracted would equal $397/950 = 0.41$. This value is identical to the weighted average for bacteria and fungi in the study of Anderson and Domsch (1978a). The data in Fig. 4B was therefore assumed to represent a true measure of the ^{14}C -carbon and ^{14}C -biomass carbon remaining in the soil during continued incubation. It is of interest to note that the conversion of $1600 \mu\text{g carbon g}^{-1}$ soil to $950 \mu\text{g biomass carbon}$ results in a growth efficiency of 59%. Extracellular

polysaccharides such as capsular material if present were considered to be a portion of the biomass.

The decay of the biomass carbon shown in a linear fashion in Fig. 4 can best be described using first order reaction kinetics. Using this procedure, day 1 of the incubation was considered as day 0 for the decay of the biomass. The decay of biomass in the control soil during incubation could be represented by a single first order decay equation (Fig. 6).

$$\text{Biomass carbon} = 662 e^{-4.5 \times 10^{-3} t}$$

The decay of unlabelled biomass carbon, in the presence of glucose yielded a curve when plotted on semilog paper; exponential regression analysis and curve splitting techniques were applied to differentiate the components. The slope of the line describing the decay of each component represents the first order net decay rate; the intercept represents the amount present. The unlabelled biomass in the glucose amended soil was separated into an active biomass accounting for 12% of the total soil biomass with a decay rate of $1.27 \times 10^{-1} \text{ day}^{-1}$. The decay rate constant for the resting biomass was $5.5 \times 10^{-3} \text{ day}^{-1}$ which approximated the decay rate constant of the biomass in the control soil.

The labelled biomass carbon could be separated into three components (Fig. 6B). Component I at a level of $328 \mu\text{g}$ carbon g^{-1} soil accounted for 40% of the glucose immobilized.

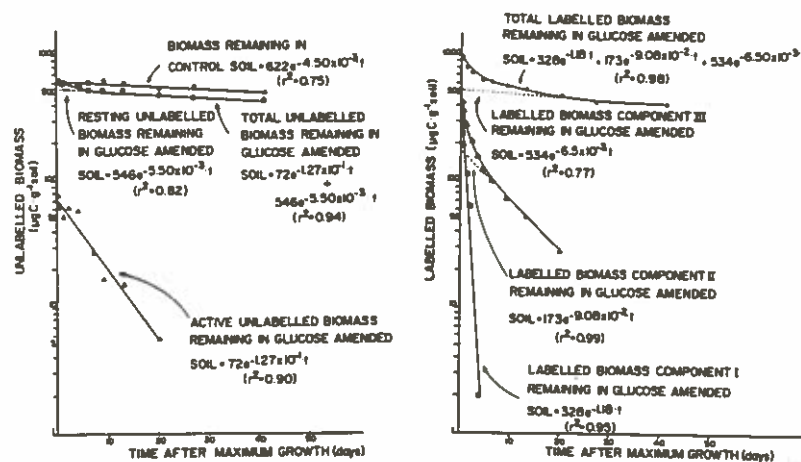


Fig. 6. Decay rates and pool sizes of the labelled and unlabelled biomass present in soil after growth on ^{14}C -glucose.

This decayed with a rate constant of 1.18 day^{-1} . Component I with a decay rate of $9.08 \times 10^{-2} \text{ day}^{-1}$ corresponded to the active unlabelled biomass. The decay rate constant of component III was $6.5 \times 10^{-3} \text{ day}^{-1}$ which is equivalent to that of the resting biomass.

The measurement of ^{14}C -biomass makes it possible to determine the production of microbial metabolites (Fig. 5). The decay of the ^{14}C -biomass during days 1 to 5 resulted in the rapid production of microbial products. They were probably largely the result of microbial death. After this period, both the total ^{14}C -carbon remaining and ^{14}C -biomass stabilized although the amount of microbial products remaining in the soil at any one time (net production) was found to decrease towards the end of the experiment.

CONCLUSIONS

The examples given for carbon and phosphorus flow through ecosystems indicate the significance of the microbial biomass as a source-sink as well as its importance in the various transformations involved. The concepts of microbial growth and activity developed from studies in pure microbiology must be applicable, perhaps with caution, to ecosystem studies. We have shown that techniques for the determination of biomass and activity exist. These together with tracer isotopes are making possible the characterization of nutrient flow in soils and sediments. However, an understanding of the behaviour of the microbial biomass within ecosystems is now limited as much by a lack of information concerning the interaction of microorganisms with their solid environment as it is by the difficulty involved in their measurement and applying microbial growth concepts to complex systems.

We have found the most useful method for determining biomass to be the CHCl_3 -fumigation incubation technique. This procedure is based on the somewhat difficult to believe observation that after lysis, bacteria and fungi are decomposed with the evolution of a relatively constant amount of CO_2 . The measurement of the biomass-C produced during *in situ* growth on added ^{14}C -glucose indicated that previous suggestions that a control CO_2 should be subtracted from the total CO_2 evolved may be in error. Our data were most easily interpreted when control CO_2 levels were not subtracted. Also it is difficult to see how a non-fumigated sample can be a control for the fumigated sample where more than 99% of the organisms have been killed. Our K_d factor measured using a labelled *in situ* population agreed with the published value of 0.41. When this value was applied during extended incubation periods and the ^{14}C -biomass subtracted from ^{14}C -carbon remaining, it was found that the

production of microbial products nearly equalled the biomass during extended incubation periods.

The separation of the unlabelled biomass into an active and dormant fraction should make possible some estimates concerning the percentage of the biomass carbon that was activated by a single pulse of glucose. The unlabelled biomass carbon, in the presence of glucose, was shown to be comprised of two fractions. One decayed at the same rate as the biomass in the unamended soil; the other decayed at the same rate as the ^{14}C -biomass produced during growth on glucose. Therefore, it should be possible to conclude that the dormant biomass represented that proportion of the biomass not activated by the glucose.

The above interpretations while indicating the potential for biomass measurements must be used with caution. All the data represented net rates. Simultaneous growth and decay were not separated. Thus only the net outcome of the two processes was measured. An estimate of the simultaneous rates of growth and decay can be obtained by mathematical simulation of the data presented in this paper in conjunction with CO_2 evolution and specific activity measurements. If the simulation accurately mimics the actual net rates for ^{14}C -carbon and ^{12}C -microbial biomass and ^{14}C -carbon and ^{12}C - CO_2 evolved, the output from the computer can be used to calculate the gross rates. The gross immobilization-mineralization rates of ^{15}N in a similar experiment were calculated by Paul and Juma (1980) to be 10 times as great as the net rates.

The techniques are now becoming available for the development of an understanding of carbon, nitrogen and even sulphur and phosphorus nutrient flow through the microbial biomass in nature. As the understanding of this system evolves, there must also be a feedback to microbial ecological studies in the laboratory. More information is required for interpretation of growth on and within soil and sediment solids. The present information on resting cells and spores also cannot explain how such a large population can persist for such extended periods without the utilization of massive carbon supplies.

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