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Turnover of Microbial Populations and their Metabolites in Soil

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Introduction

Soil is the expression of the interaction between the biosphere and the lithosphere under the control of the abiotic factors of moisture and temperature. A knowledge of the ecology of organisms within this complex milieu is fundamental to our understanding of natural ecosystems and of energy flow through them. Although energy is packaged and transported as ATP in biological systems, most is originally derived from the oxidation of carbonaceous substrates by heterotrophic organisms. Measurement of the production of CO_2 during metabolism of these substrates is a useful monitor of the energy production within soil systems. We must therefore develop techniques to relate data obtained from measurements of this parameter to turnover of organic residues and microbial populations. The effect of C supply on mineral N availability has long been recognized (Pinck, Allison & Gaddy, 1946; Bartholomew, 1965). It is essential that the relationships between C and N turnover rates be quantified.

The use of CO_2 as an index of activity requires that we be able to separate plant root respiration from microbial respiration in soil and that C/N ratios and efficiencies of in situ populations be known. To this end a series of integrated studies were initiated at Saskatoon as part of the International Biological Programme — Matador Project. The following describes their applicability to other soil systems.

Project Framework

Objective	Approach
Separate plant root and microbial respiration	Pulse label photo-synthesizing plants
Determine C and N mineralization rates from plant materials	Add labelled plant materials to field soils
Determine rate of metabolism of defined compounds in field and laboratory soils	Add labelled glucose or acetate and $(NH_4)_2\ SO_4$ to soils and incubate
Develop a fractionation technique to study turnover of biomass and metabolites in situ	Pyrophosphate extraction followed by sonication
Develop a conceptual model of the soil sub-system using above results	Using above data, calculate quantities of metabolites and amount of C and N turnover

C & N Turnover in the Field

Plant materials (mature wheat and native grasses) labelled with ^{14}C and ^{15}N were added to virgin and cultivated soils and allowed to undergo biological transformations for up to three years. This permitted the study of decomposition rates of the added material with subsequent estimates of biomass production and of humification under natural conditions. Results of these investigations (Shields & Paul, 1973; McGill & Paul, 1973a) indicated that synthesized microbial metabolites were more resistant to decomposition than a significant portion of the initially added plant residues. Similar characteristics for both C and N turnover through microbial metabolites were observed in the soils investigated. This indicates that turnover of organic N in soil is intimately related to C turnover, and that C is the driving force for these transformations.

In a study using ^{14}C labelled glucose rather than plant material under field conditions on a clay soil Shields et al. (1973) observed that microbial metabolites produced from the glucose were very stable. This stabilized C may be present as a large inactive biomass since partial sterilization with $CHCl_3$ followed by reinoculation and incubation released 30 per cent of the residual ^{14}C in 14 days. Freezing and thawing and wetting and drying released only 16.2 per cent and 7.9 per cent respectively (Shields, Paul & Lowe, 1973). Measurements of numbers of microorganisms indicated that both fungal and bacterial populations developed simultaneously (Fig. 2). Under the conditions of this investigation less of the added C was evolved as CO_2 during 14 days (44 per cent) than is observed under similar conditions in the laboratory in which 70-80 per cent may be evolved during this length of incubation period (Fig. 3). Also, under field conditions very little N was remineralized; under laboratory conditions, 30 per cent or more may be remineralized in a comparable period. These data suggest that in the field system under study, in which both the bacterial and fungal populations had stabilized prior to substrate addition, both populations tend to function in a commensalistic manner with a consequent lack of recycling of metabolites. The glucose utilization efficiency of approximately 60 per cent, is the same as that reported in a review by Payne (1970). Under laboratory conditions, using the same soil, but acetate as a substrate the added C was not stabilized until all but 30 per cent of the added C was evolved.

Laboratory Studies

The flow of carbon and nitrogen through the soil subsystem and the apparent discrepencies in the efficiency of carbon utilization may be explained in light of intensive studies on this soil under laboratory conditions. In addition studies such as this add immensely to our information on and understanding of the functioning of the plant-soil-microorganism system. ^{14}C-acetate and ^{15}N-ammonium sulphate were used as substrates in soil which had previously been air dried. An initial development of fungi followed by bacteria and actinomycetes was expected (Kornberg & Elsden, 1961). Thus, the effect of sequential population developments and of competition could be studied.

The added acetate-C was rapidly metabolized with a half-life of 3-4 days (Sorensen & Paul, 1971; McGill, Shields & Paul, 1973) which is similar to that for glucose-C under field conditions. Nitrogen was rapidly immobilized and mineral N reached a minimum by day 4-5 (Fig. 1) followed by net mineralization for up to 100 days. Carbohydrates, amino

acids, and amino sugars were rapidly synthesized and reached a maximum at the same time as mineral N levels reached a minimum. These synthesized materials were degraded more slowly than the original substrate. The half-life of amino acid -C left in the soil after 90 days (2,748 days) was longer than for the amino acid -N (1,600 days), thus demonstrating a gradual recycling of N with concomitant loss of C as CO_2.

Labelled C and N released by sonication reached a maximum at the same time as did amino acids, amino sugars and carbohydrates. Particulate material (cell wall components and some ribosomes) continued to accumulate for a slightly longer period indicating greater resistance to attack in this fraction than in cytoplasmic materials. Turnover of carbon and nitrogen through the various fractions was highly correlated, lending support to the premise that organic N turnover is dependent on C or energy availability.

The fungal population developed prior to the bacterial population. This sequential population development resulted in a more competitive microbial environment with greater turnover of microbial metabolites than was observed under field conditions (Fig. 4).

Development and Testing of a Model

Although data on numbers of fungi and bacteria, CO_2 production and nitrogen mineralization were available they did not provide quantitative information on the turnover of microbial biomass during degradation of organic substrates in soil. Turnover of organic -C and N in soil depends on production and destruction of microbial tissue and secondary extracellular products. A mathematical description of the relationships existing between C and N turnover through soil microorganisms was compiled to described the conceptual model (Fig. 5) developed after consideration of the data from the laboratory and field investigations. The model was designed to fit the laboratory system since two populations had been separated out and CO_2 evolution data were available on a daily basis.

The main concepts apparent after examining the data and which were used in developing the model of the system are briefly listed as (McGill & Paul, 1973c);

1. Two biochemically separate populations developed sequentially.
2. The primary population may be defined as that population which was the sink for acetate-C but which also assimilated more complex substrates.
3. The secondary population was defined as that population which utilized microbial metabolites and soil organic matter but not acetate-C.
4. All populations were assumed to undergo some cryptic growth.
5. The quantity of C and N entering a given population was a function of the biomass-C.
6. The quantity of C or N released from a given population was dependent on the amount present.
7. N turnover was strictly dependent upon C turnover.
8. The relative proportions of soil-C and labelled-C (or N) in a population could be determined using the specific activity and quantity of evolved CO_2 as the experimental input to the model.
9. Plate count data were used to provide an index of microbial activity with 100 per cent activity being considered to occur when the plate count numbers reached an upper plateau. This plateau was observed

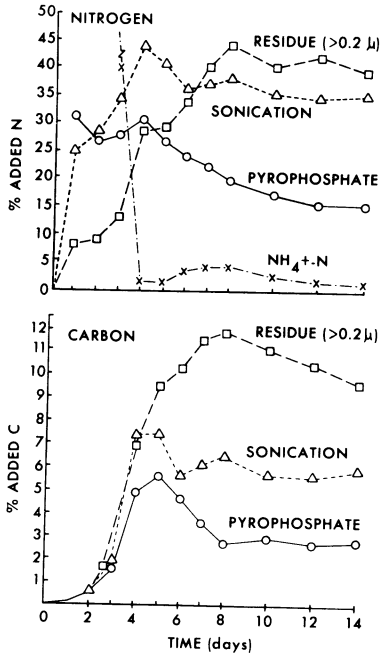


Fig. 1

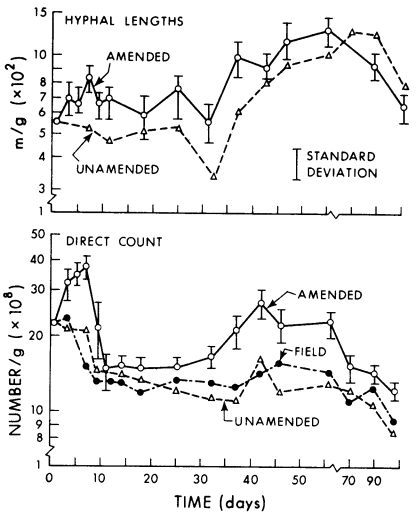
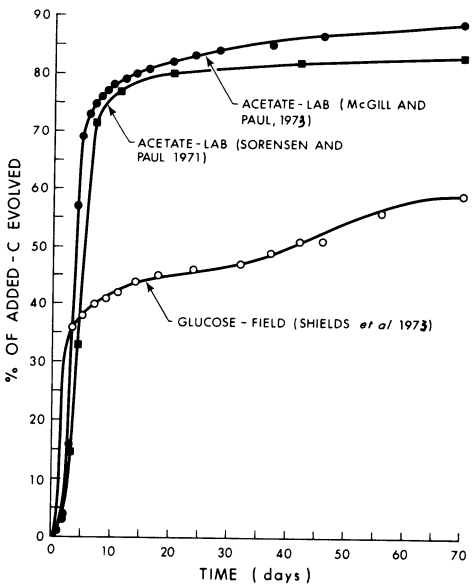


Fig. 2



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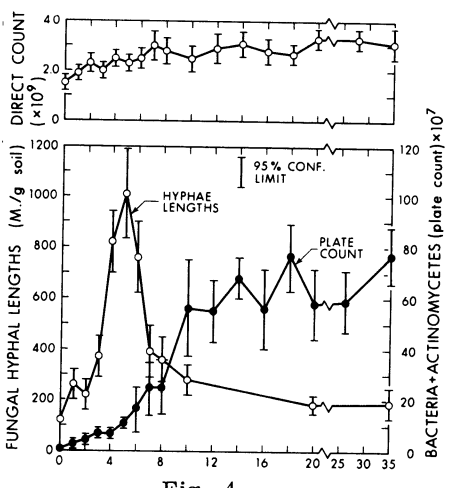


Fig. 4

during the period after day 10 (Fig. 4). The values obtained from the plate count prior to day 10 (numbers, biomass, C or N) were then calculated as a per cent of that value. These percentage values were then multiplied by the direct count values (total population) to obtain a quantitative estimate of the viable bacterial biomass.

Carbon dioxide evolution rates and specific activities were used as experimental input to the model. To check the fit of the calculations and constants used, the predicted mineral N levels and atom per cent excess ^{15}N values were compared with experimental values obtained during the laboratory incubation from which the CO_2 evolution data were acquired (Fig. 6).

The model predicted mineral N and ^{15}N levels in accordance with experimental data. Thus it was felt that the concepts and mathematical relationships used to develop the model were essentially correct and that the model is a reasonable approximation of C and N turnover in the soil system under investigation.

The laboratory data indicate that carbon had become relatively stabilized when 30 per cent or less remained. This value in the past has been assumed to represent the efficiency of utilization of added-C. The model output indicated that C was utilized by both populations with an efficiency of 60 per cent. This efficiency value is in accordance with field results and results reported for microorganisms in aqueous culture (Payne, 1970). The low apparent efficiency is due to rapid recycling of C within the laboratory system which did not stop until 70-80 per cent of the C had been evolved. In the field, where the population was stabilized and little turnover of metabolites occurred, the C loss was only 40-50 per cent. The field system was primarily one in which the added substrate was utilized to produce new biomass and metabolites, which were stabilized as viable cells and as material adsorbed to soil colloids. In the laboratory, the shift in population resulted in a much more competitive system in which metabolites were reutilized rapidly and in which active predation prevented metabolite adsorption to soil colloids from significantly reducing turnover rates.

Fig. 1 Labelled carbon and nitrogen in fractions isolated from Sceptre soil incubated at 18°C after addition of acetate -^{14}C (4000 μg C/g) and $(^{15}NH_4)_2 SO_4$ (145 μg N/g).
Residue — that fraction having a particle size > 0.2 μ and separated after extraction of the soil with pyrophosphate.
Sonication — that fraction released by sonication after extraction with pyrophosphate and having a diameter < 0.2 μ.
Pyrophosphate — that fraction solubilized by 0.1M sodium pyrophosphate.

Fig. 2 Bacterial numbers and fungal hyphae lengths in Sceptre field soil — either amended with ^{14}C labelled glucose (937 μg C/g) or unamended.

Fig. 3 Labelled CO_2-C evolved from Sceptre soil under field and laboratory conditions after addition of labelled substrate.

Fig. 4 Numbers of bacteria and actinomycetes and fungal hyphae lengths in Sceptre soil incubated at 18°C after addition of acetate -^{14}C (4000 μg C/g) and $(^{15}NH_4)_2SO_4$ (145 μg N/g).

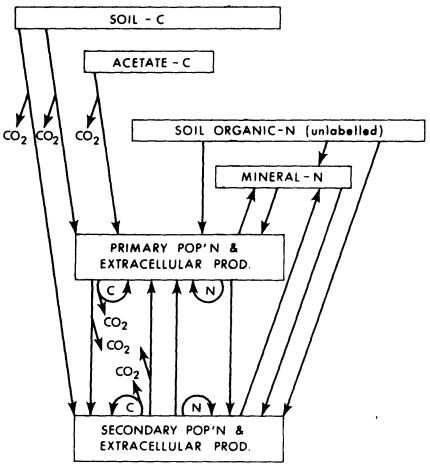


Fig. 5

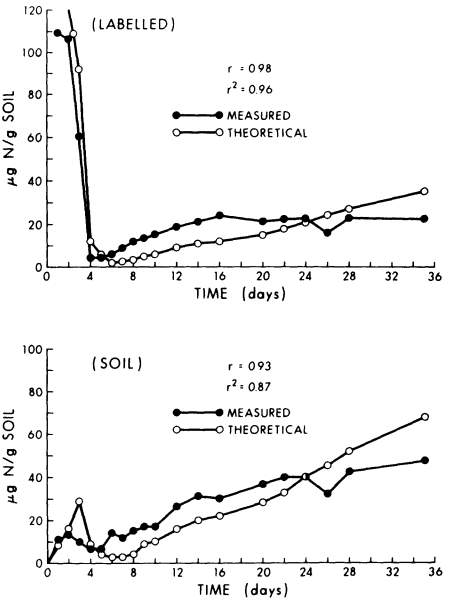


Fig. 6

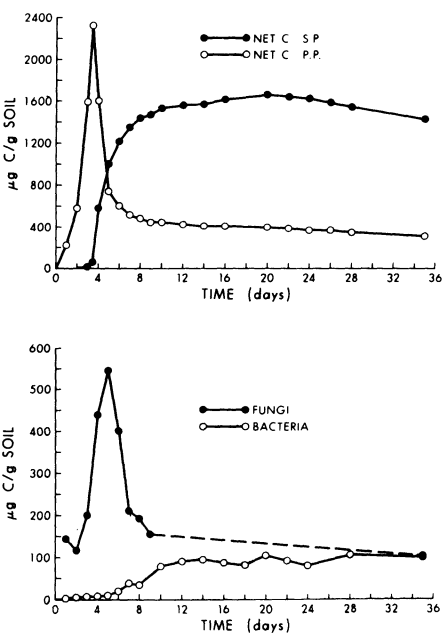


Fig. 7

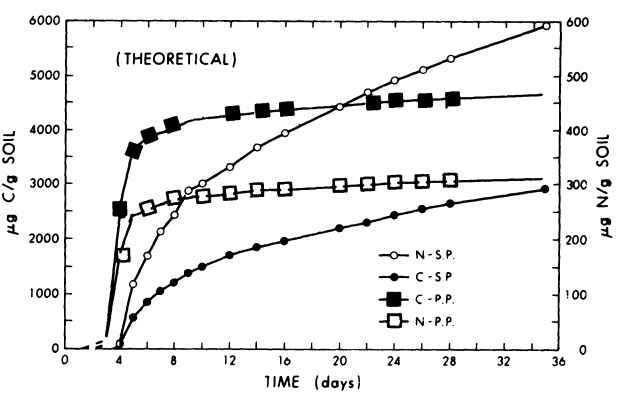


Fig. 8

Simulation of the laboratory system also indicated that the primary population had a C/N ratio of 15 which was 3 times that of the secondary or bacterial population. This high C/N ratio is reflected in various chemical parameters, notably the labelled C/N ratios of isolated microbial fractions (McGill, Shields & Paul, 1973) C/N ratios of hydrolyzable labelled materials and in the shorter half-life for C than for N in amino acids (Sorensen & Paul, 1971; McGill & Paul, 1973b). The shift in microbial population from one with a high C/N ratio to a lower one is consistent with the gradual reduction in labelled C/N ratios of these various fractions.

The soil organic matter or unlabelled C and N that was utilized by these microbial populations had a C/N ratio of 15. Therefore, much of the microbial attack was on recently incorporated roots and plant litter which was not removed prior to incubation. The rapid turnover of these substrates relative to more humified material has also been observed in the field (Jenkinson, 1971; McGill & Paul, 1973a; Shields & Paul, 1973).

Parameters of utmost importance in the plant-microorganism-soil system and which are almost impossible to measure directly are:

1. the quantity of C and N cycled through various soil populations and
2. the quantity of extracellular material and lytic products produced.

Computer simulation of the system using labelled substrates together with direct measurements of biomass provide means by which this can be achieved.

In the laboratory where 4000 μg of labelled C were added per gram of soilaand 3042 μg labelled C and 1770 μg soil C were evolved by day 8, 1922 μg were present as identifiable microbial biomass and metabolites (Fig. 7). An additional 5296 μg of soil and labelled C had undergone at least one cycle of synthesis and decomposition (Fig. 8).

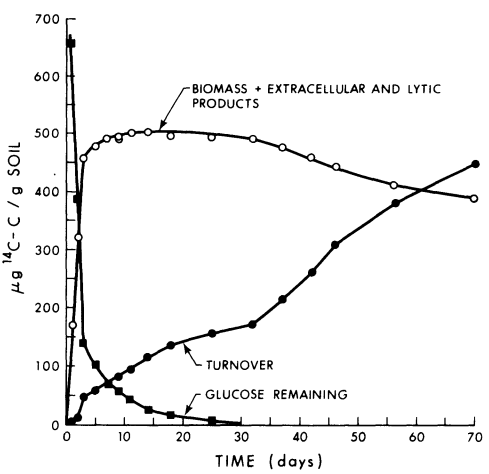
Fig. 5 Conceptual model of C and N turnover through various soil and microbial components.

Fig. 6 Mineral-N levels (labelled and soil) in Sceptre soil incubated at 18°C after addition of acetate -^{14}C (4000 μg C/g) and $(^{15}NH_4)_2SO_4$ (145 μg N/g) predicted by the model (Fig. 5) and measured in the soil.

Fig. 7 Carbon contents of microbial compartments of Sceptre soil incubated at 18°C following addition of acetate -^{14}C (4000 μg C/g) and $(^{15}NH_4)_2SO_4$ (145 μg N/g).
Net C S.P. — Model's prediction of the C in the secondary population (Fig. 5).
Net C P.P. — Model's prediction of the C in the primary population (Fig. 5).
Fungi — C in fungal hyphae calculated on basis of hyphae lengths reported in Fig. 4.
Bacteria — C in bacteria and actinomycetes calculated from data reported in Fig. 4.

Fig. 8 Carbon and nitrogen turnover (using the model in Fig. 5) through microbial populations of Sceptre soil incubated at 18°C after addition of acetate -^{14}C (4000 μg C/g) and $(^{15}NH_4)_2SO_4$ (145 μg/g).

Thus, about 3 times as much material had been synthesized and reutilized as substrate, as remained in the microbial pool (viable biomass plus extracellular and lytic products). However, calculations based on labelled C data from field sites indicated that after 11 days of field incubation, for every unit of labelled C remaining in microbial biomass and metabolites, only 0.2 units were turned over. This value reached 1.2 after 70 days incubation in the field (Fig. 9). In the laboratory the fungal or primary population was more active in C turnover than was the secondary population, whereas the reverse was true for N. With this type of data the differences in field and laboratory results can be explained on the basis of amount of recycling of metabolites and lytic products. It also becomes evident from these data and from direct microscopic examination of the soil that only about 10-30 per cent of the total pool of microbial material could be accounted for as identifiable biomass using FITC (fluorescein isothiocyanate). FITC quantitatively stains bacteria but does not give quantitative data on the older fungal population or large spheres such as protozoa. The rest was present as extracellular metabolites, dead cells and lytic products (Fig. 10). Thus, there is very little reason to expect a decline in microbial populations under most natural conditions even though labelled C and N may continue to be mineralized. The pool of available metabolites is large enough to supply sufficient substrate to buffer the system against rapid losses in microbial biomass itself as a result of predation. Hence, the frequently observed phenomenon of very stable microbial populations occurs under both laboratory and field conditions in the presence of C and N mineralization from sources considered to be of microbial origin. Changes in these populations can then be expected only after significant increases in subtrate levels or following periods of lethal stress.



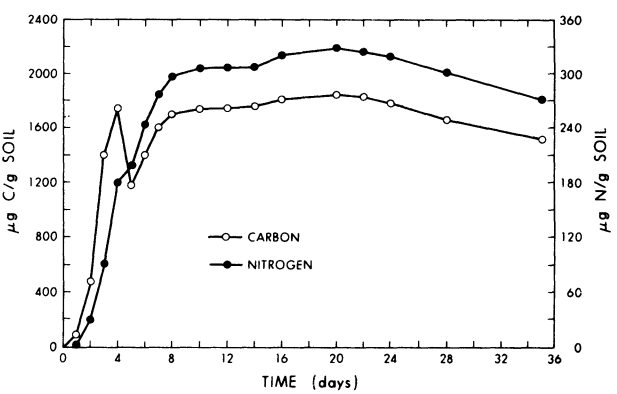


Fig. 9 Fate of added carbon under field conditions as predicted by the model (Fig. 5) using evolved CO_2 as the experimental data input.

Fig. 10 Extracellular carbon and nitrogen in Sceptre soil incubated at 18°C after addition of acetate -^{14}C (4000 μg C/g) and $(^{15}NH_4)_2SO_4$ (145 μg N/g) as predicted by the model (Fig. 5).

This type of integrated approach in which the flow of substrate C and N can be traced through soil based on an easily measured parameter such as CO_2 evolution, and in which both chemical and microbiological measurements are made, facilitates a more complete definition of the system and a better understanding of energy and nutrient cycling through it. The use of CO_2 evolution as the primary driving force for modelling allows data from studies which separate soil and root respiration to be utilized fully in describing C and N cycling through the soil system. Data from investigations into decomposition rates of plant litter can also be included and the turnover of microbial metabolites during this process can thus be quantified. However, no one universal model is likely to be applicable, each system must first be carefully characterized chemically and microbiologically and from these data a conceptual model and finally a mathematical model developed.

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