

RELATION BETWEEN CARBON AND NITROGEN TURNOVER IN SOIL ORGANIC FRACTIONS OF MICROBIAL ORIGIN

W. B. MCGILL,* J. A. SHIELDS† and E. A. PAUL

Saskatchewan Institute of Pedology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0

(Accepted 20 May 1974)

Summary—Labelled ^{14}C -acetate and ^{15}N - $(\text{NH}_4)_2\text{SO}_4$ were added to a clay soil in the laboratory to follow transformations of microbial C and N. A fungal population developed initially, reaching a maximum by day 5, then rapidly declined and was replaced by a population dominated by bacteria and actinomycetes. Soil samples containing doubly-labelled microorganisms and their metabolites were extracted by $\text{Na}_4\text{P}_2\text{O}_7$, and the extracted material further separated with phenol.

The highly labelled acid-soluble (fulvic acid) fraction of the $\text{Na}_4\text{P}_2\text{O}_7$ extract contained extracellular metabolites of low molecular weight which were rapidly attacked and converted to new microbial biomass, metabolites, mineral N or CO_2 . $\text{Na}_4\text{P}_2\text{O}_7$ also removed an acid-insoluble (humic acid) fraction of which up to 70 per cent of the labelled C and N could be removed by phenol. Attack of these recently synthesized extracellular materials was indicated by a rapid decline of $\text{Na}_4\text{P}_2\text{O}_7$ extractable C and N during the growth of bacteria and actinomycetes.

Following $\text{Na}_4\text{P}_2\text{O}_7$ extraction, the residue was sonicated and peptized in water and the components of the microbial biomass were partitioned into sedimentation fractions by centrifugation. The components concentrated in the $>0.2\ \mu\text{m}$ fraction, which were hypothesized as being cell wall components, were more resistant to attack than materials in the $<0.04\ \mu\text{m}$ fraction. The materials in the latter fraction were thought to originate from cytoplasmic constituents. The labelled materials in the $<0.04\ \mu\text{m}$ sized fraction, which accumulated as the fungal population developed, were utilized less rapidly by the developing bacterial population.

Decomposition of the microbial population resulted in transfer of C and N through various sediment fractions. The organic fraction (considered to be cytoplasmic material and adsorbed extracellular metabolites) which became labelled as the bacterial population developed, was utilized less rapidly by the developing bacterial population than components removable by $\text{Na}_4\text{P}_2\text{O}_7$. Evolution of $^{14}\text{CO}_2$, production of microbial material and immobilization of N closely paralleled the incorporation and release of these elements from the fractions. The similarity of the behavior patterns of these elements suggested they were intimately associated within the soil microbial system studied. This demonstrated that N transformations were highly dependent on C transformations.

INTRODUCTION

The relationship between C and N turnover in soil is governed primarily by the energy supply within the system and the stability of plant residues and microbially derived materials that are produced from substrate utilized by a growing microbial population. Jansson (1958, 1960) demonstrated a mineralization-immobilization cycle of N in soil and suggested that organic matter could be separated into a small active fraction and a large passive fraction. In establishing principles involving the use of labelled microbial tissue for studying the transformations of organic C, he emphasized that the added energy source must be rapidly and completely transformed into microbial materials or CO_2 leaving no residue of the original

substrate. It has been shown that ^{14}C -tagged glucose (Persson, 1968) and acetate (Ivarson and Stevenson, 1964; Sørensen and Paul, 1971) meet these requirements thereby permitting microbial populations to be labelled in the soil environment. Labelled C is distributed among different organic fractions after short periods of incubation (Mayaudon and Simonart, 1958; Ivarson and Stevenson, 1964; Mutatker and Wagner, 1967; Persson, 1968).

Microbial cells labelled in culture have been added to soil (Mayland and McIntosh, 1966; Knowles and Chu, 1969). Living cells added to non-sterile soil did not persist suggesting the cells were unable to adapt to their new environment. Mayaudon and Simonart (1963) studying the humification of added labelled bacterial cells and fungal mycelium observed that *Azotobacter* cells were mineralized faster than *Aspergillus*. Hurst and Wagner (1969) reported hyaline organisms underwent more rapid decomposition than melanic organisms, with the cell-wall fraction of a

* Present Address: Department of Soil Science, University of Alberta, Edmonton, Alberta T6G 2E1.

† Present Address: Soil Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario K1A 0C6.

melanic fungus being more resistant to decomposition than the cytoplasm or the wall and cytoplasm fractions from hyaline organisms (Wagner, 1968).

Different fractions of organic N are known to mineralize at different rates but little is known of the chemical nature of the fraction dominated by readily mineralizable N (Keeney and Bremner, 1966; Freeney and Simpson, 1969; Stewart *et al.*, 1963). Reagents capable of selectively extracting mineralizable forms of soil N were studied by Stanford (1968) who reported that more total N was extracted by $\text{Na}_4\text{P}_2\text{O}_7$ than by CaCl_2 . However, the amounts of distillable N removed from soils by these reagents were of similar magnitude. Tracer investigations have suggested the biological transformations of C and N are tied closely together with labelled soil C being more closely related to organic N than to total organic C (Simonart and Mayaudon, 1966; Martel, 1972). However, the role of C in controlling turnover of N through the organic fractions has not been defined.

The objective of this investigation was to determine the nature of microbial populations developed *in situ* following the addition of substrate containing labelled C and N to the soil system. The distribution of microbially produced organic materials including extracellular metabolites was followed by a fractionation technique and the relationship between C and N was evaluated. This integrated approach made it possible to study the turnover of C and N in relation to microbial population dynamics.

MATERIALS AND METHODS

The soil used for this study was taken from the surface horizon of undisturbed grassland at the Matador Project site for the International Biological Programme. This virgin Sceptre soil was developed under short grass prairie vegetation (*Stipa comata*, *Koeleria cristata*, *Agropyron dasytachyum*) on fine textured, calcareous, lacustrine deposits in the Brown soil zone (Mitchell *et al.*, 1944) southeast of Kyle, Saskatchewan. The total C was 3.0 per cent; inorganic C was 0.10 per cent; total N 0.27 per cent; total clay 59 per cent; pH 7.8. The soil was stored in the air-dry state for one month before incubation.

Addition of labelled substrates

Three kilograms of air-dry soil were passed through a 4 mm sieve. Sufficient ^{14}C -labelled acetate (100 $\mu\text{Ci/g}$ C) and ^{15}N -labelled ammonium sulfate (5.206 at. % excess) to supply 4 mg C and 145 μg N/g soil were dissolved in sufficient water to bring the soil to 40% moisture by weight (90 per cent of field capacity). The soil and substrate were mixed and allowed to equilibrate for 12 h at 2°C. The amended soil was incubated at 18°C in a large desiccator fitted with an aeration train to collect evolved $^{14}\text{CO}_2$. Soil samples were removed at regular intervals and dried at 60°C. At the beginning of the incubation period, three portions of soil (50 g dry wt) were removed, placed in 250 ml Erlenmeyer

flasks and connected to an aeration train to measure the labelled and unlabelled CO_2 evolved. Evolved CO_2 was trapped by bubbling through 0.2 N NaOH contained in a 500 ml flask fitted with a column (600 \times 10 mm) filled with 3 mm glass beads. Activity (^{14}C) of the evolved CO_2 was measured on 1.0 ml samples of the NaOH by counting in a Triton mixture (Shields and Paul, 1973).

Fractionation

Soil (20 g) was shaken for 2 h with 0.01 N H_2SO_4 (200 ml) to remove carbonates. It was then stirred for 20 h (under N_2) with 200 ml 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$, centrifuged and the extraction repeated two more times. This extract was fractionated by acidifying (2 N H_2SO_4) to pH 1.7. The precipitate removed by centrifugation was dissolved by adjustment to pH 8.5 with 0.5 N NaOH, reprecipitated with H_2SO_4 (pH 1.7) and dissolved again in NaOH (pH 8.5); this fraction was termed humic acid (HA). The decantate from the humic acid precipitation was combined with the initial 0.01 N H_2SO_4 extract and termed fulvic acid (FA).

The residue of $\text{Na}_4\text{P}_2\text{O}_7$ extraction was mixed vigorously with 200 ml distilled water, sonicated for 10 min at 125 W with a Biosonik II and the suspension allowed to stand for 48 h (Anderson *et al.*, 1974) before centrifugation (1000 g for 30 min). The residue contained particles greater than 0.2 μm . The suspended material was further separated according to particle size by centrifugation (12,000 g for 60 min). The material containing particles from 0.2 to 0.04 μm dia was termed fine clay (FC). Dissolved and suspended material in the decantate (<0.04 μm) was separated according to its sensitivity to precipitation by acid (pH 1.7). The precipitate removed by centrifugation was termed fraction-B (Fr-B); the acid-soluble portion was called fulvic acid-B (FA-B).

The humic acid was fractionated with phenol (Biederbeck and Paul, 1973) as outlined below. Ten millilitres of 75% phenol (pH 5.0) and 5 ml of HA (500 μg N) solution were added to 50 ml polyethylene centrifuge tubes and shaken for 20 min at room temperature. After centrifugation (12,000 g for 10 min), the tubes were allowed to sit for 10 min before the organic phase containing phenol was removed from the bottom of the tube with a syringe. The extraction was repeated two more times. The composite phenolic extract was adjusted to pH 8.0 and transferred to a Majonnier flask containing an equal volume of anhydrous diethyl ether. This was hand shaken, allowed to stand for 10 min and the ether phase containing phenol was decanted and discarded. The ether extraction was repeated until a negative test for phenol (0.5 N FeCl_3) was obtained in the extract. Ether was removed from the remaining solution by rotary evaporation under vacuum and the solution was made to volume. This fraction was termed phenol extract.

All extractions were conducted at room temperature. The large volume of fulvic acid (FA) was dried by vacuum evaporation. All fractions were made up to

volume and stored at 5°C. Before C determination, aliquots were transferred into combustion flasks and freeze-dried.

Analytical methods

Total carbon and radioactivity were determined (Shields and Paul, 1973). Total nitrogen was determined by the semimicro Kjeldahl method and ammonium and nitrate by steam distillation. An Atlas Model GD 150 mass spectrometer was used for the determination of ^{15}N (Johns, 1971).

Measurements of microbial numbers

Total bacteria were counted using fluorescein isothiocyanate (Babiuk and Paul, 1970). Viable bacteria were counted on a 10^6 serial dilution of soil using soil extract agar as a growth medium with a 14-day incubation period (Babiuk and Paul, 1970). Fungal hyphae lengths were measured in $10\ \mu\text{l}$ samples of a 10^2 dilution of soil spread over a $1\ \text{cm}^2$ area of a microscope slide, allowed to dry and stained with fluorescein isothiocyanate. An eyepiece grid was calibrated using a stage micrometer slide. One hundred and fifty fields of each smear were examined and the length of fungal hyphae in each field was determined using the calibrated eyepiece grid.

RESULTS

Respiration, microbial numbers and mineralization of C and N

Only a small amount of the added C was respired during the first 3 days (Fig. 1) with the maximum rate occurring between day 3 and day 4. Nearly 70 per cent of the added C had been evolved after 5 days and 80 per cent by the end of 14 days. Accumulation of labelled C in the organic fractions followed a pattern similar to that for C respired, with most of the accumulation occurring between the 3rd and 4th days.

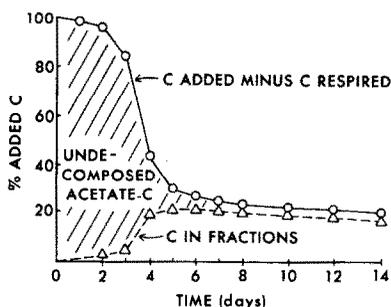


Fig. 1. Fate of labelled C in virgin Sceptre soil amended with ^{14}C -acetate (C in fractions = $\sum \text{C}$ extracted by $\text{Na}_4\text{P}_2\text{O}_7$, released by sonication and contained in the soil residue).

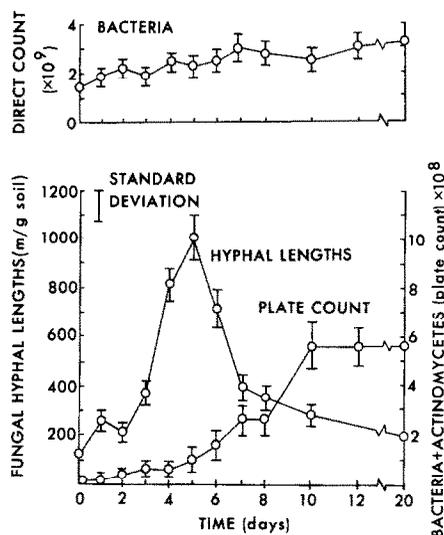


Fig. 2. Numbers of bacteria and lengths of fungal hyphae/g Sceptre soil incubated at 18°C after addition of ^{14}C -acetate and ^{15}N - $(\text{NH}_4)_2\text{SO}_4$.

Microbial growth was negligible during the first 2 days (Fig. 2). The first population to develop was predominantly fungal reaching a maximum (1000 m hyphae/g) by day 5 after a 10-fold increase in hyphal lengths. Decline of the fungal population was accompanied by a rapid increase in numbers of viable bacteria to 6×10^8 organisms. The number of bacteria as measured by the direct count method doubled during this period. After 14 days, the number of bacteria measured by plate count and direct count methods did not decline.

After the initial lag period rapid immobilization of added N occurred and by day 4 less than 3 per cent remained in the mineral form (Fig. 3). Thereafter, net N mineralization of $20\ \mu\text{g/g}\ \text{NO}_3^- \text{N}$ suggested degradation of recently synthesized microbial constituents during growth of the secondary microbial population and nitrification of the NH_4^+ (data not shown).

Fractionation of soil organic matter by the sodium pyrophosphate sonication technique

The main difference between the data for C and N in various fractions of soil organic matter is shown by the high amount of labelled N in the $\text{Na}_4\text{P}_2\text{O}_7$ extract during the first 3 days (Fig. 3). This was attributed to large amounts of added mineral- NH_4^+ recovered during that period. After day 3, added mineral N remaining in the soil was negligible and the labelled N present in the $\text{Na}_4\text{P}_2\text{O}_7$ extract resembled the pattern displayed by C for that fraction. Nearly all the undecomposed labelled acetate-C extracted by the initial acid

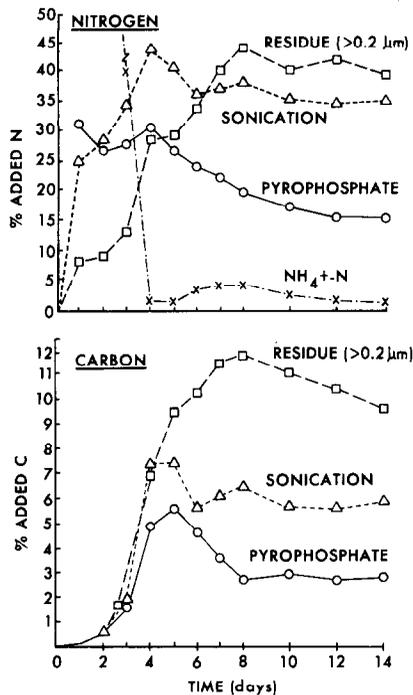


Fig. 3. Labelled organic C and N in fractions isolated by $\text{Na}_4\text{P}_2\text{O}_7$ and sonication.

pretreatments (which was combined with the FA fraction) was removed during subsequent vacuum evaporation of the FA fraction and therefore would not appear in Fig. 3. The low boiling point (118.5°C) and highly volatile nature of added acetate- ^{14}C facilitates its removal from solutions of the organic fractions by

evaporating or freeze-drying. Labelled C remaining after this treatment is of microbial origin.

Sonication released (or dispersed) additional organic and inorganic materials. The patterns for labelled C and N released were similar ($r = 0.70$, $P < 0.05$) increasing rapidly to a maximum on day 4 before declining to slightly lower concentrations (Fig. 3). A highly significant correlation coefficient ($r = 0.97$, $P < 0.01$) was obtained between C and N present in the $>0.2 \mu\text{m}$ residue. The amounts of labelled materials in this fraction increased steadily during the first 8 days in contrast to the maximum observed after 4 days in materials extracted by $\text{Na}_4\text{P}_2\text{O}_7$ or sonication.

The amount of radioactive C in the fulvic acid (FA) and humic acid (HA) and the phenol-soluble component of the HA increased during the first 5 or 6 days, declined slightly until day 8 and then remained stable (Table 1). Labelled N in HA showed a similar pattern to C ($r = 0.93$, $P < 0.01$) reaching a maximum on day 4. The high values of N for FA during the first 3 days suggest that this fraction contained the added mineral-N previously mentioned. However, after day 3 when mineral-N was depleted, the amount of labelled N present in this acid soluble component was still 2.3 times that in the precipitable HA. Phenol extracted three-quarters of the labelled C and one-half of the N present in the HA fraction (Table 1). This supports the hypothesis that much of the microbial material associated with HA is not covalently bonded. Phenol which breaks hydrogen bonds has been used to selectively separate humoproteins from soil (Biederbeck and Paul, 1973).

Material released during sonication was further separated into three fractions (Table 2). Most of the labelled organic C released was isolated in the very fine ($<0.04 \mu\text{m}$) dissolved and suspended material of the fraction-B (Fr-B) which accounted for 6 per cent of

Table 1. Labelled C and N in fractions of organic material extracted by sodium pyrophosphate and further fractionated with phenol

Day	Carbon (% of added C*)			Nitrogen (% of added N†)			Ratio phenol ext: HA		Ratio C:N	
	FA	HA	Phenol extract	FA	HA	Phenol extract	C	N	HA	Phenol extract
1	0.4	0.1	N.D.	30.4	1.0	N.D.	N.D.	N.D.		
2	0.5	0.1	N.D.	24.9	2.3	N.D.	N.D.	N.D.		
3	0.9	0.7	0.5	22.6	5.2	1.9	0.72	0.37	3.6	7.2
4	2.1	2.8	2.2	21.9	9.7	5.2	0.79	0.54	7.7	11.6
5	2.3	3.2	2.5	18.3	8.5	4.1	0.78	0.48	10.2	16.5
6	2.4	2.2	1.7	18.6	5.8	2.7	0.77	0.47	10.2	17.1
7	2.1	1.4	1.0	16.9	5.5	2.4	0.71	0.44	6.9	11.3
8	1.5	1.2	0.8	14.7	5.2	2.3	0.67	0.44	6.3	9.4
10	1.8	1.1	0.8	12.3	5.0	2.6	0.73	0.52	6.1	8.3
12	1.5	1.1	0.8	11.8	4.3	2.1	0.73	0.48	6.9	10.5
14	1.6	1.1	0.7	11.7	4.3	1.6	0.64	0.37	6.9	11.6

* 4 mg C/g.

† 145 μg N/g.

N.D. not determined.

Table 2. Labelled C and N in organic fractions extracted by sonication and peptization of the residue from the $\text{Na}_4\text{P}_2\text{O}_7$ extraction

Day	Labelled C (% of added C*)			Labelled N (% of added N†)		
	Fr-B ($<0.04 \mu\text{m}$)	FA-B	Fine Clay ($0.2-0.04 \mu\text{m}$)	Fr-B ($<0.04 \mu\text{m}$)	FA-B	Fine Clay ($0.2-0.04 \mu\text{m}$)
1	0.1	0.1	0.1	10.9	5.8	8.3
2	0.4	0.1	0.1	11.2	8.1	8.6
3	1.5	0.3	0.1	16.7	9.2	8.5
4	6.0	0.8	0.5	24.8	11.2	8.2
5	6.2	0.7	0.5	23.1	11.0	7.2
6	4.5	0.9	0.2	19.8	10.8	6.3
7	5.1	0.7	0.3	21.5	10.1	5.7
8	5.4	0.7	0.3	22.7	9.5	6.1
10	4.6	0.7	0.3	20.5	8.2	6.6
12	4.5	0.6	0.4	21.3	7.2	6.3
14	4.7	0.7	0.4	22.7	8.0	6.1

* 4 mg C/g.

† 145 mg N/g.

added-C after 4 days. Fulvic acid-B (FA-B) and fine clay ($0.2-0.04 \mu\text{m}$) fractions consistently contained only a small proportion of the total labelled C in the sonication extract. Following sonication, the largest proportion of released, labelled N was also isolated in Fr-B, amounting to 25 per cent of added N after 4 days, before stabilizing at a slightly lower amount (Table 2). The remaining labelled N was nearly equally divided between the FA-B and fine clay (FC) fractions with the labelled N component being much higher than the C in these fractions. A relatively high amount of labelled N occurred in the sonication extractable components during the first 3 days suggesting a portion of the added mineral N was adsorbed to mineral colloids associated with these materials.

Except for FC and $>0.2 \mu\text{m}$ residue, the maximum specific activity of the fractions (Fig. 4) occurred between the 3rd and 4th days when fungi were most abundant and most C was being respired. The activity of FC and $>0.2 \mu\text{m}$ residue continued to increase during the growth period of the secondary population. The highest degree of labelling occurred in FA-B.

DISCUSSION

Sequential development and turnover of material of a doubly labelled microbial population within a soil environment was demonstrated by microbial measurements and by fractionation of the soil organic matter. The qualitative nature of the microflora was influenced by addition of acetate-C (Kornberg and Elsdén, 1961) and the use of stored air-dry soil which selectively stimulated the fungal population. Autolysis of the initial fungal population or its attack and decomposition by a second population (bacteria and actinomycetes) is indicated by the net mineralization of labelled $\text{NH}_4^+\text{-N}$ between days 4 and 8. In contrast, Shields *et al.* (1973)

reported the immediate growth of both bacterial and fungal populations upon addition of glucose and NH_4NO_3 to moist soil under field conditions.

In this study, it was observed that added C and N was incorporated into all the organic fractions isolated. The degree of incorporation closely paralleled the observed production of microbial tissue (plate counts and lengths of fungal hyphae). Both C and N

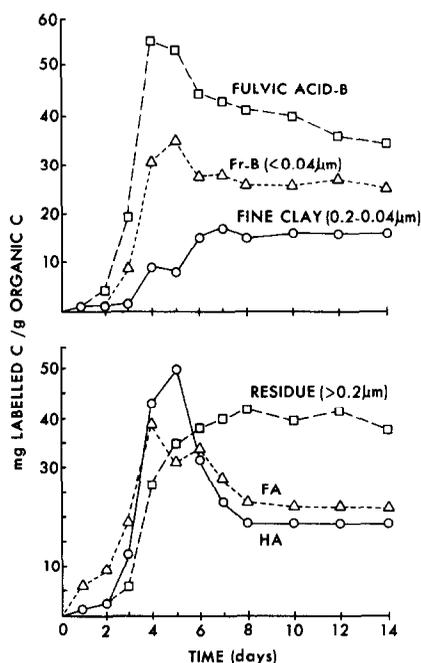


Fig. 4. Specific activity of soil fractions isolated by $\text{Na}_4\text{P}_2\text{O}_7$ and sonication.

could be incorporated into these organic fractions only through microbial metabolic activity. The net result is that microbial materials (extracellular products, cytoplasmic materials or cell walls and other particulate components) were partitioned among the various organic entities separated out by the fractionation system used. The question then arises as to the mode of partitioning that one would expect to occur.

Anderson *et al.* (1974) showed that the molecular weight range of HA (humic acid) was lower than that for fraction B (extracted after sonication and peptization). They also found higher C/H ratios and greater hydrolyzability of C and N in fraction B than HA. This indicates that fraction B is more aliphatic and contains fewer condensed aromatic components than HA extracted by $\text{Na}_4\text{P}_2\text{O}_7$. The lower coagulation threshold values for HA reported by Anderson *et al.* (1974) further supports this conclusion. McGill (1971) found that 50 per cent of the amino acid-N in a clay soil and 40% in a sandy soil were present in fraction-B. The lower C/N ratio of fraction-B, its high molecular weight and high amino acid content indicate that much of the N in fraction-B is proteinaceous in nature. Conversely the material contained in HA contains substantial quantities of condensed aromatic structures. These materials are not highly hydrolyzable and generally have a lower molecular weight range than found in fraction-B. Since HA is extracted by $\text{Na}_4\text{P}_2\text{O}_7$, its occurrence in soil must be in association with polyvalent cations. It is also removed from soil before sonication indicating that labelled materials in this fraction were extracellular components. Results obtained by phenol extraction of HA indicate that these low molecular weight HA materials were associated with the organic colloids by a weak bonding system, possibly a H-bonding system.

Biederbeck and Paul (1973) showed that HA contained a large proteinaceous moiety which could be removed with phenol. In the present study, the C/N ratios of labelled material extracted from HA with phenol indicate that it must contain a carbonaceous component in addition to the protein moiety. The C/N ratio reached a maximum of 17:1 when fungi predominated but declined as bacteria became dominant. McGill *et al.* (1973) reported that computer simulation of this system predicted a C/N ratio of 15:1 for materials synthesized during this period of fungal dominance.

The rapid decline in labelled C and N extracted by $\text{Na}_4\text{P}_2\text{O}_7$ during the growth of the secondary population demonstrates the utilization of extracellular products as substrates for further microbial activity. Fulvic acid is characteristically lower in molecular weight and more highly oxidized than HA with which it is extracted (Felbeck, 1971). The loss of labelled C and N from FA demonstrates the rapid transformation of both C and N from this fraction by successive populations of soil microorganisms. FA is often considered the final step in humification (Schnitzer, 1967). The presence of highly labelled FA produced within 3–6

days after addition of labelled acetate or $(\text{NH}_4)_2\text{SO}_4$ indicates that the fulvic fraction also contains substantial quantities of very young materials.

After ultrasonic treatment, separation of cytoplasmic materials and cell walls is generally accomplished by centrifugation; cell walls settling out more rapidly than ribosomes. The soluble cytoplasmic material remains in solution. Ultrasonic vibration of an aqueous soil suspension can be expected to have the same effect on microorganisms as it does in other aqueous systems. Soil contains a very large adsorbing surface in the very fine clay fractions (fraction-B). Therefore, materials which would be found with the soluble cytoplasmic component, we suggest, would be adsorbed onto the surface of clay and hence would be found in fraction-B. This is consistent with the highly proteinaceous nature of fraction-B and with the observation of McGill (1971) that N incorporation into fraction-B parallels N incorporation into amino acids and amino sugars.

Fraction-B also is expected to contain extracellular enzymes. The large molecular weight of fraction-B (Anderson *et al.*, 1974) and its high amino acid content (McGill, 1971) further support this conclusion. Therefore, we suggest that labelled materials in fraction-B represent extracellular enzymes, high molecular weight lytic products and soluble cytoplasmic materials released during ultrasonic vibration.

It is suggested that the more rapidly settling sedimentation fractions contain the particulate materials, such as ribosomes and cell walls. Hurst and Wagner (1969) concluded that cell walls are likely to accumulate in soil organic matter. In the present study the label in the particulate material ($>0.2 \mu\text{m}$ size fraction) continued to increase until day 8 demonstrating a greater stability of labelled C and N in this fraction than material containing the cytoplasmic or extracellular constituents which reached a maximum by day 4.

During the period between day 4 and days 8–10, low molecular weight extracellular material may have served as substrate with some contribution from the higher molecular weight constituents of fraction-B. Growth of a second population resulted in a net increase in the labelled C and N content of the residue ($>0.2 \mu\text{m}$). By day 10, the labelled N and C content of the FA fraction (low molecular weight extracellular constituents) had been depleted and cell synthesis, CO_2 evolution and N mineralization proceeded slowly. A gradual reduction in C and N in the residue occurred thereafter due to its use as a substrate.

Carbon and nitrogen turnover in this soil through the various organic fractions were intimately related. The highly significant correlation coefficients of 0.93, 0.97, 0.97 between C and N in HA, Fr-B and $>0.2 \mu\text{m}$ respectively, demonstrate that N transformations through these fractions is highly dependent on C transformations.

Acknowledgements—The valuable technical assistance of M. Akister and L. Johns is gratefully acknowledged. Apprecia-

tion is also extended to Dr. J. N. Ladd for helpful suggestions and critical review of the manuscript. This paper is issued as Saskatchewan Institute of Pedology Publication No. R107.

REFERENCES

- ANDERSON D. W., PAUL E. A. and ST. ARNAUD R. J. (1974) Extraction and characterization of humus of Saskatchewan soils, with particular reference to clay associated humus. *Can. J. Soil Sci.* **54**, 317-324.
- BABIUK L. A. and PAUL E. A. (1970) The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. *Can. J. Microbiol.* **16**, 57-62.
- BIEDERBECK V. O. and PAUL E. A. (1973) Fractionation of soil humate with phenolic solvents and purification of the nitrogen-rich portion with polyvinylpyrrolidone. *Soil Sci.* **115**, 357-366.
- FELBECK G. T. (1971) Structural hypothesis of soil humic acids. *Soil Sci.* **111**, 42-48.
- FREENEY J. R. and SIMPSON J. R. (1969) The mineralization of nitrogen from soil organic fractions in soils. *Soil Biol. Biochem.* **1**, 241-251.
- HURST H. M. and WAGNER G. H. (1969) Decomposition of ^{14}C -labelled cell wall and cytoplasmic fractions from hyaline and melanin fungi. *Proc. Soil Sci. Soc. Am.* **33**, 707-711.
- IVARSON K. C. and STEVENSON I. L. (1964) The decomposition of radioactive acetate in soil—II. The distribution of radioactivity in soil organic fractions. *Can. J. Microbiol.* **10**, 667-682.
- JANSSON S. L. (1958) Tracer studies on nitrogen transformations in soil with special attention to mineralization-immobilization relationships. *Ann. Royal Agric. Coll. Sweden* **24**, 101-361.
- JANSSON S. L. (1960) On the establishment and use of tagged microbial tissue in soil organic matter research. *Trans. Seventh Int. Congress of Soil Science, Madison, Wisconsin* **2**, 635-642.
- JOHNS L. E. (1971) Mass Spectrometry. In *Isotope Methodology and Techniques in Soil-Plant Nutrition and Plant Physiology*, pp. 19-42. Saskatchewan Institute of Pedology Publication No. 76.
- KEENEY D. R. and BREMNER J. M. (1966) Characterization of mineralizable nitrogen in soils. *Proc. Soil Sci. Soc. Am.* **30**, 714-719.
- KNOWLES R. and CHU D. T. H. (1969) Survival and mineralization of ^{15}N -labelled *Serratia* cells in a boreal forest raw humus. *Can. J. Microbiol.* **15**, 223-228.
- KORNBERG H. L. and ELSDEN S. R. (1961) The metabolism of 2-carbon compounds by microorganisms. *Adv. Enzymol.* **23**, 401-470.
- MCGILL W. B. (1971) *Turnover of Microbial Metabolites During Nitrogen Mineralization and Immobilization in Soil*. Ph.D. Thesis, University of Saskatchewan, Saskatoon, Canada.
- MCGILL W. B., PAUL E. A., SHIELDS J. A. and LOWE W. E. (1973) Turnover of microbial populations and their metabolites in soil. In *Modern Methods in the Study of Microbial Ecology* (T. Rosswall, Ed.), *Bull. Ecol. Res. Comm. (Stockholm)* **17**, 293-301.
- MARTEL Y. (1972) *The Use of Radiocarbon Dating for Investigating the Dynamics of Soil Organic Matter*. Ph.D. Thesis, University of Saskatchewan, Saskatoon, Canada.
- MAYAUDON J. and SIMONART P. (1958) Etude de la decomposition de la matiere organique dans le sol, au moyen de carbone radioactif. II. Decomposition du glucose radioactif dans le sol. *Pl. Soil* **9**, 376-380.
- MAYAUDON, J. and SIMONART P. (1963) Humification des microorganismes marques par ^{14}C dans le sol. *Ann. Inst. Pasteur* **105**, 257-266.
- MAYLAND H. F. and MCINTOSH T. H. (1966) Distribution of nitrogen fixed in desert algal crusts. *Proc. Soil Sci. Soc. Am.* **30**, 606-609.
- MITCHELL J., MOSS H. C. and CLAYTON J. S. (1944) *Saskatchewan Soil Survey Report No. 12* pp. 73-76. University of Saskatchewan, Saskatoon, Canada.
- MUTATKER V. K. and WAGNER G. H. (1967) Humification of carbon-14 labelled glucose in soils of Sanborn Field. *Proc. Soil Sci. Soc. Am.* **31**, 66-70.
- PERSSON J. (1968) Biological testing of chemical humus analysis. *LantbrHogsk. Ann.* **3**, 81-217.
- SCHNITZER M. (1967) Humic-fulvic acid relationships in organic soils and humification of the organic matter of these soils. *Can. J. Soil Sci.* **47**, 245-250.
- SHIELDS J. A., LOWE W. E., PAUL E. A. and PARKINSON D. (1973) Turnover of microbial tissue in soil under field conditions. *Soil Biol. Biochem.* **5**, 753-764.
- SHIELDS J. A. and PAUL E. A. (1973) Decomposition of ^{14}C -labelled plant material in soil under field conditions. *Can. J. Soil Sci.* **53**, 297-306.
- SIMONART P. and MAYAUDON J. (1966) Etude des transformations de la matiere organique du sol au moyen du carbone-14. In *The Use of Isotopes in Soil Organic Matter Studies*, pp. 245-258. *FAO/IAEA Technical Meeting*, 1963. Pergamon Press, Oxford.
- SORENSEN L. H. and PAUL E. A. (1971) Transformation of acetate carbon into carbohydrate and amino acid metabolites during decomposition in soil. *Soil Biol. Biochem.* **3**, 173-180.
- STANFORD G. (1968) Effect of partial removal of soil organic nitrogen with sodium pyrophosphate or sulfuric acid solutions on subsequent mineralization of nitrogen. *Proc. Soil Sci. Soc. Am.* **32**, 679-682.
- STEWART B. A., PORTER L. K. and JOHNSON D. D. (1963) The availability of fertilizer nitrogen immobilized during decomposition of straw. *Proc. Soil Sci. Soc. Am.* **27**, 656-659.
- WAGNER G. H. (1968) Significance of microbial tissues to soil organic matter. In *Isotopes and Radiation in Soil Organic Matter Studies*, pp. 197-205. *FAO/IAEA*, Vienna.