TURNOVER OF MICROBIAL BIOMASS, PLANT RESIDUES AND SOIL HUMIC CONSTITUENTS UNDER FIELD CONDITIONS

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Abstract

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The effects of soil texture and climatic conditions on turnover rates of plant residues were measured under field conditions. Carbon-14- and ^{15}N-labelled straw made it possible to follow degradation rates of the original substrate and of the soil organic constituents formed during the initial degradation process. Subsequent sampling measured the turnover of the active fraction. Carbon dating was used to measure the turnover rates of the more resistant fraction. Fractionation of the soil during the first two years showed greater accumulation of a condensed aromatic moiety (humic acid A) in the medium-textured Luvisolic soil and in the coarse-textured Dark Brown Chernozemic (Kastanozem). High clay grassland soils showed protection of aliphatic nitrogen from further humification. Much of the initial nitrogen and carbon mineralization of soil organic materials produced on decomposition of the straw came from the fulvic acids which contained a predominance of recently synthesized low molecular weight materials. Carbon and nitrogen incorporation into the > 0.2 μm fraction lagged behind incorporation into other fractions. Large quantities of immobilized carbon and nitrogen were contained in the > 0.2 μm fraction as well as in the 0.04 μm sedimentation fraction allowing these two fractions to act as sources of slowly released nitrogen. Residual humic acid carbon and nitrogen turnover was best estimated from carbon dating of the carbon after fractionation of the soil. The nitrogen turnover was calculated utilizing the C/N ratios of the fractions. Acid hydrolysis was found to be the simplest method of fractionation of large quantities of soil for carbon dating and for specific components. $Na_4P_2O_7$ extraction followed by peptization and sediment analysis proved useful for measuring C and N transformations on a shorter term basis.

INTRODUCTION

Measurement of N transfers from plant residues through the soil biomass to humic constituents and mineral products involves a variety of tracer techniques and fractionation methods. An understanding of N transfer also involves an understanding of C transformations, for N flow and stabilization is dependent on transformations of organic C. In our laboratory, C flow has been measured by direct microscopy of bacteria and fungi [1,2], CO_2 evolution, enzyme assays and measurement of the flow of ^{15}N and ^{14}C through specific compounds [3,4]. This paper brings together data for tracer and fractionation techniques used to follow the C and N through soil organic fractions of Chernozemic and Luvisolic soils. The plant residue decomposition study utilized ^{14}C/^{15}N labelled straw and ^{15}N-NO_3 plus unlabelled straw to compare the effect of soil texture and climatic zone on the active soil organic fraction. Carbon dating of virgin and cultivated sites was utilized to investigate the turnover of resistant humic materials.

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TABLE I. MEAN RESIDENCE TIMES AND $\delta^{14}C$ OF THE FRACTIONS OF THE BROWN
CHERNOZEMIC SOIL (SCEPTRE) AND DISTRIBUTION OF TOTAL N, ORGANIC C AND
^{14}C IN THE FRACTIONS AS IT OCCURS IN NATURE AND 350 DAYS AFTER THE
ADDITION OF LABELLED ^{14}C-ACETATE

	Radiocarbon-dated soil Ap 0–10 cm[a]					Incubated soil Ah 0–15 cm[b]	
	MRT	$\delta^{14}C$	N	^{14}C	Soil-C	^{14}C	Soil-C
	y B.P. ± 1σ	‰ ± 1σ	% of total			% of total	
Total soil	350 ± 65	−43 ± 9	100	100	100	100	100
Light material	Modern	+243 ± 18[c]	3.0	8.0	6.0	5.0	5.0
0.5N hydrolyzate[c]	Modern	+7 ± 15	41	37	35	49	35
6N hydrolyzate[c]	Modern	+61 ± 22	43	24	22	21	20
6N residue	1 765 ± 65	−197 ± 16	13	31	37	25	40
NaOH extract	1 910 ± 105	−212 ± 16	9.0	22	27	16	23
Water extract	1 790 ± 120	−200 ± 15	1.5	2.6	3.1	3.4	7.0
Humin	1 330 ± 100	−153 ± 100	2.5	6.4	6.9	5.6	10.0

[a] Carbon content 1.9%; nitrogen content 0.20%.

[b] Carbon content 2.1%.

[c] Data were calculated by difference.

CARBON DATING

On carbon dating a Sceptre, clay grassland soil, it
was found that the mean residence time (MRT) for the total
soil after water flotation was 350 yr B.P. (Table I).
$ZnBr_2$ with a specific gravity of 2.0 g/cm^3 floated off a
fraction with a $\delta^{14}C$ of +243 $^o/oo$. This comprised 6% of
the C and 3% of the soil N for a C/N ratio of 17. Micro-
scopic observations indicated that it was comprised of
partly decomposed plant materials [5]. The high ^{14}C
content is attributable to nuclear bomb explosions. The
presence of this material in the soil had the same effect
on the total MRT as would 16% modern C from pre-bomb plant
materials. The high level of ^{14}C in the atmosphere makes
it possible to obtain a higher level of accuracy in tracer
C work using low background methods. It also affects all
future carbon dates of materials, such as soil, which are
in equilibrium with the environment.

The $\delta^{14}C$ values for the 0.5 N and 6 N HCl hydrolyzates
were +7 $^o/oo$ and 61 $^o/oo$ respectively. Removal of 0.5 N
hydrolyzable C which accounted for 37% of the total C
resulted in an MRT of 855 years for the residue. The
residue after 6 N hydrolysis was 1765 years. Further
fractionation released most of the C and N with MRT
ranging from 1330 to 1910 years.

TABLE II. MEAN RESIDENCE TIMES AND CHARACTERISTICS
OF THE ORGANIC MATTER IN THE PROFILE OF THE VIRGIN
SCEPTRE SOIL

Horizon depth (cm)	Ah (0–10)		C-A (10–20)	Ck (20–30)	
	%	MRT	%	%	MRT
Total carbon (%)	2.6	545	1.5	1.3	1325
Nitrogen (%)	0.29	–	0.18	0.14	–
Hydrolyzable-C[a]	60	195	65	67	–
Hydrolyzable-N[a]	87	–	89	89	–
Residue	40	1100	–	–	–

[a] Percentage of total soil content.

The organic matter of the equivalent virgin site
(Table II) had a somewhat higher MRT than the cultivated,
probably indicating less incorporation of recent
vegetation with high levels of ^{14}C attributable to
nuclear bomb explosions. The MRT of the residue after
acid hydrolysis of the virgin site was twice that of the
total C. In the cultivated site, the residue of
hydrolysis had an MRT five times that of the total C.
Cultivation for 20 years resulted in a 20% reduction in
total C [6]. Equal proportions of C (60%) and N (86%)
were hydrolyzed in both samples, although the MRT's were
different.

LABORATORY STUDIES

In a previous investigation, soil samples containing
doubly labelled microorganisms and their metabolites were
extracted by $Na_4P_2O_7$ and the extracted material further
separated by phenol. The sediment fraction was sonicated
and peptized in water and partitioned by centrifugation [7].
The components, concentrated in the >0.2 μm fraction, which
were hypothesized as being cell wall debris, were more
resistant to attack than materials in the <0.04 μm fraction.
Fulvic materials showed high turnover rates. The similarity
of the behaviour pattern for the C and N verified that they
were intimately associated [8].

The ^{14}C content of the field soil measured by radio-
carbon dating was compared with the ^{14}C distribution after
amendment with ^{14}C acetate and 350 days incubation in the
laboratory and fractionation (Table I). The laboratory-
labelled soil accumulated 70% of the ^{14}C substances in the
hydrolyzable fractions compared to the 61% for the carbon-
dated soil. This difference in the ^{14}C distribution came
from the material removed by 0.5 N HCl hydrolysis which

TABLE III. NITROGEN BALANCE SHEET (4 years) FOR FERTILIZER N ADDED TO BRADWELL AND SUTHERLAND SOILS

			Bradwell		Sutherland	
			112N	112N + Straw	112N	112N + Straw
			(kg/ha)			
1967–68	Plant	1967	16.4	11.8	44.4	51.6
		1968	7.0	10.1	10.6	5.4
	Roots		1.4	1.3	1.2	1.0
	Soil	0–15 cm	21.5	31.0	15.9	28.0
	(1968)	15–30 cm	8.0	6.1	7.2	4.6
		30–90 cm	19.8	12.8	4.5	3.0
	Total recovered		74.1	73.1	83.8	93.6
	Original added, 1967		112.0	112.0	112.0	112.0
	% Recovery		66	65	75	84
1969–70	Plant	1969	0.8	1.2	0.7	1.0
		1970	0.6	0.9	0.3	0.5
	% of that added, 1969		1.2	1.2	1.1	0.9
	Soil	0–15 cm	12.5	16.7	9.7	17.5
		15–30 cm	0.6	1.4	1.8	1.2
		30–90 cm	1.3	1.9	0.8	0.5
	Total recovered		15.8	22.2	13.3	20.6
	Original added, 1969		17.7	25.9	13.2	22.6
	% Recovery		89	86	101	91

extracted 12% more ^{14}C from the incubated soil. The distribution of N was more closely associated with the ^{14}C materials (r = 0.86) than the distribution of soil C (r = 0.77).

STRAW DECOMPOSITION

Use of ^{15}N-NO_3 plus unlabelled straw or dual labelling of straw in the growth chamber before placement in the field, made it possible to follow the relationship between C and N in the $Na_4P_2O_7$ extract and in the sediment fractions of field soils. In two of the soils, a Bradwell fsl and a Sutherland clay, the N was incorporated as fertilizer N with unlabelled straw [9]. Organic ^{15}N components isolated were the result of microbial growth and immobilization of ^{15}N. The coarse textured Bradwell had poor plant N uptake under a moisture deficit. It showed high mineral N values especially at depth, and also showed high N losses (Table III).

In 1968, the 0-15 cm layer plus straw contained 31 kg/ha of organic ^{15}N compared to 21 kg in the treatment without straw, indicating a net immobilization of 10 kg/ha

TABLE IV. TOTAL ORGANIC ^{14}C AND ^{15}N REMAINING IN SOIL AFTER STRAW ADDITION

Period	% of added			
	1	2	3	4
^{14}C				
Sceptre	40	41	28	26
Waitville	36	25	23	18
^{15}N				
Sceptre	55	67	52	47
Waitville	55	49	43	35

in this layer. Plant uptake of the immobilized N was low in the third and fourth year after N immobilization, with less than 1% of the original ^{15}N being removed by the plants per year. However, the straw plots with immobilized ^{15}N showed a 50% greater ^{15}N uptake.

The largest drop in N and C occurred during the first period of investigation before fractionation (Table IV). The organic ^{15}N remaining in the Sceptre soil was 54% of the original added at the time of the first fractionation after one period of incubation which was equivalent to one-half of a six-month growing season. During the remainder of the period, the N content dropped another 7%.

The ^{14}C in the dually labelled straw added to field soils dropped more rapidly than the ^{15}N with the Luvisolic soils showing higher loss rates than the Chernozemic. Thirty-five percent of the ^{15}N and 18% of the ^{14}C remained in the Waitville soil, whereas the Sceptre still contained 47% of the ^{15}N and 26% of the ^{14}C after two years in the field.

Fractionation of the six soils showed that the largest portion of the ^{15}N was in the >0.2 μm fraction (Fig. 1 and 2), with the two heavy clay soils giving similar results even though the original source of the ^{15}N was different. These materials with a ^{14}C/^{15}N ratio of 15 for the Sceptre and 12 for the Waitville (Table V) tended to accumulate with time.

The fulvic acids were the most active for they showed the largest percentage decrease. The release of C from this fraction was not as great as the release of N during the second and third incubation periods resulting in an increased C/N ratio (Table V). The <0.04 μm fraction showed a slight increase in percentage during the second period of incubation with a consequent drop in the amount of material present,
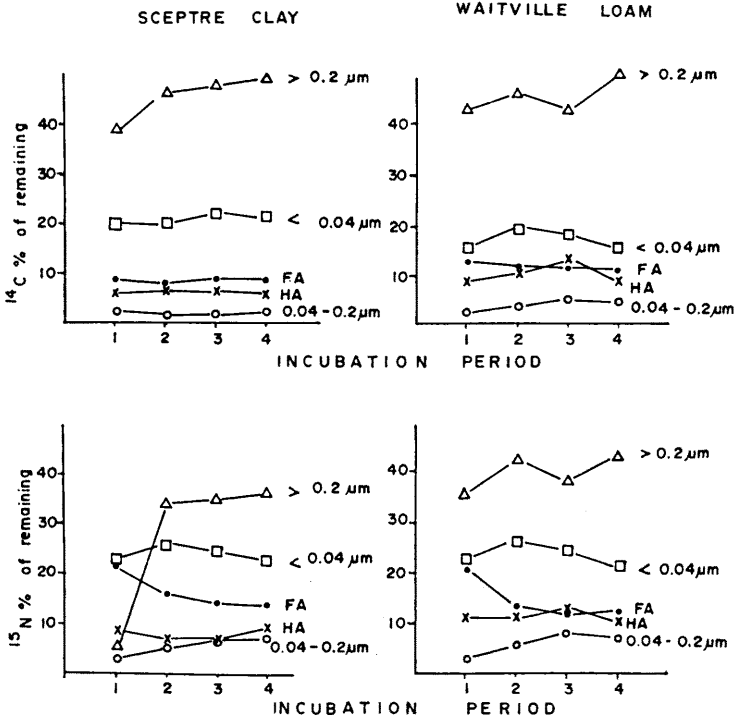
FIG.1. Labelled C and N distribution in fractions of Sceptre and Waitville soil amended with $^{14}C\text{-}^{15}N$ straw.
Each incubation period represents 3 months of a 6-month growing period.
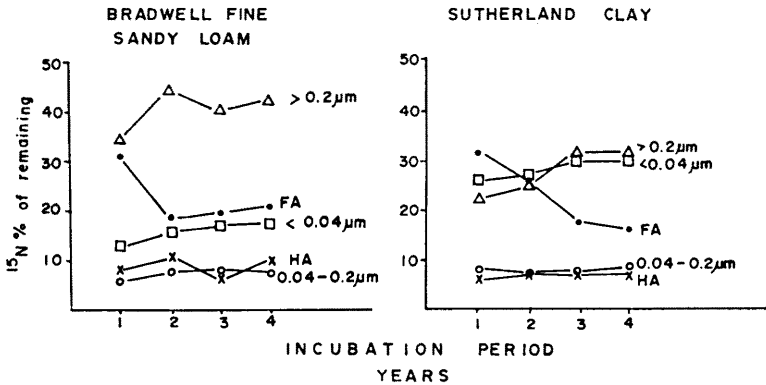

FIG.2. Labelled N distribution in Bradwell and Sutherland soils amended with $^{15}N\text{-}NO_3$ and unlabelled straw.

TABLE V. LABELLED AND NON-LABELLED C/N RATIOS OF FRACTIONS OF
SCEPTRE AND WAITVILLE SOILS

	C/N unlabelled soil	$^{14}C/^{15}N$ of labelled soil			
		Period of incubation			
		1	2	3	4
		Sceptre			
FA	6.7	6.2	6.2	7	7.5
HA	9.8	9.9	11.5	10.0	8
<0.04 μm	9.2	10.6	9.0	8.9	8.4
0.04–0.2 μm	4.5	6	3.6	4	4.2
>0.2 μm	15.6	–	16.8	15.3	14.6
		Waitville			
FA	8.7	7.75	9.0	10.4	7.5
HA	9.5	8.6	11.5	7.9	9.8
<0.04 μm	8.3	9.2	7.8	8.2	7.8
0.04–0.2 μm	6.8	7	5.7	6.8	7.5
>0.2 μm	11.9	15.6	11.6	12.2	8.3

indicating a fairly large proportion of the labelled flow
through this fraction. Table V also indicates that the
largest sized fraction (>0.2 μm) which tends to accumulate
materials also had the widest C/N ratios for both labelled
and unlabelled materials.

DISCUSSION

     No one fractionation system can be expected to fully
characterize the turnover rates of soil organic constituents.
Acid hydrolysis was shown [5] to effectively remove "active"
C, both from 350-day incubated ^{14}C samples in the laboratory
and for carbon dating of natural samples in the field.

     Simonart and Mayaudon [10] also have reported that
when ^{14}C glucose, hemicellulose or cellulose decomposed in
soils, the ^{14}C and the soil N were similarly distributed.
This suggests that the active fraction is associated with
the N, and can be analyzed using ^{15}N and non-hydrolytic
techniques. The resistant materials are best investigated
using C either in long term field experiments or carbon
dating. Acid hydrolysis, because it removes a large portion
of the amino acid N not normally available to microorganisms,
does not result in separate nitrogenous fractions which are
meaningful from a turnover standpoint. The turnover of N
must be calculated from the turnover of C using data for
the C/N ratio of the various fractions [6].

The fractionation using 0.1 N $Na_4P_2O_7$ followed by sonication and peptization in H_2O, yield a humic acid fraction and a sedimentation fraction which differed markedly in degree of hydrolyzability, ^{15}N content and amino acid N content [7]. It is suggested that the non-hydrolytic technique based largely on dispersion of the inorganic-organic colloids and analysis of the sediment, can be used to interpret the fate of microbiologically immobilized N compounds in the soil. Materials removed by 0.1 M $Na_4P_2O_7$ are associated with polyvalent cations in the soil. Materials such as cytoplasmic constituents released from the biomass would be expected to be adsorbed to inorganic colloids. They should be concentrated in the <0.04 μm fraction. Cell wall and other particular debris would be expected to appear in the larger sized sedimentation fractions which showed a lag in the build-up of both tracer C and N, and accounted for the largest percentage of tracer in the soil system. This was the case both in soils incubated with mineral N and radioactive glucose or non-radioactive straw, or when ^{14}C/^{15}N labelled straw was added to the system and allowed to decompose.

The large molecular weight of the <0.04 μm fraction [11] and its high amino acid content [7] suggest that labelled materials in this fraction represent extracellular enzymes, high molecular weight lytic products and soluble cytoplasmic materials released during ultrasonic vibration. Ahmad and Harada [12] concluded that the principle form of organic N contributing to mineralization was amino acid N, and that a major origin of amino acid N contributing to the mineralization process might be peptide complexes such as muco-peptides and structural proteins which originate from microbial cell walls. Considering the dominance of amino acid N in plant and microbial systems and the previous work with ^{15}N regarding this material, the conclusions for amino acid N are not difficult to support.

The fractionation technique employed will depend on the purpose of investigation. A combination of NaOH or $Na_4P_2O_7$ with sonication and peptization, together with acid hydrolysis of the more specific components will probably yield the most meaningful results.

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DISCUSSION

D.R. SAUERBECK: I am astonished at your high recovery of up to 100% of the applied mineral fertilizer nitrogen. In our experiments we found losses of more than 40% during the first few winter months in an unplanted soil. Of course, this was in an area where the soil seldom gets frozen.

E.A. PAUL: The 100% recovery came from a soil amended with ^{15}N straw and incubation in the field under continuous cropping. The nitrogen was utilized by the plants as it was mineralized. Under fallow, unplanted soil conditions we often get losses of 15—20% during the first year in the field.

J.N. LADD: In your experiment on the decomposition of immobilized ^{15}N in a field soil, you showed that after four years the percentage of residual labelled nitrogen accounted for in the particle size fraction > 0.2 μm was approximately 40%. How does this percentage compare with the proportion of unlabelled soil nitrogen in this fraction? Some studies we have carried out over shorter periods indicate that the silt size fraction in particular may account for an increasing proportion of the more resistant nitrogenous residues.

E.A. PAUL: Our data show an accumulation with time of both ^{14}C and ^{15}N in this fraction, with a gradual lowering of the ^{14}C/^{15}N ratio. After four years, the labelled nitrogen percentage was similar to the unlabelled. Initially more ^{14}C than ^{15}N enters this fraction.