FRACTIONATION OF SOIL AND ^{15}N NITROGEN TO SEPARATE THE ORGANIC AND CLAY INTERACTIONS OF IMMOBILIZED N

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Labelled ^{15}N was added to two soils in cylinders in the field, and allowed to equilibrate for two summers of crop growth. The labelled soils were fractionated to provide information on the effect of organic and inorganic colloids on the stabilization of immobilized ^{15}N. Organic materials removed by 0.5 N NaOH without pretreatment contained more ^{15}N than those extracted by the same reagent following decalcification and removal of sesquioxides with dithionite and HCl. Both extracts had similar amino acid (contents) and similar degrees of hydrolyzability. A fractionation system using an initial 0.1 M NaOH–0.1 M $Na_4P_2O_7$ extraction followed by sonication and peptization in H_2O yielded a humic acid fraction and a sedimentation fraction (< 0.04 μm) which differed markedly in degree of hydrolyzability, ^{15}N content and amino acid-N content. The N associated with inorganic colloids < 0.04 μm, and that remaining in solution after the removal of larger particles accounted for 50% of the amino acid-N in a clay soil, and 40% in a fine sandy loam soil. Removal of sesquioxides followed by a second 0.5 N NaOH extraction reduced the N content of the colloidal size fractions of both soils, indicating that amorphous iron and aluminum compounds on the surface of clays are probably the active agents in bonding organic N to inorganic colloids. It is suggested that the nonhydrolytic technique, based largely on dispersion of the inorganic–organic colloids and analyses of the sediment, could be used to interpret the fate of microbiologically immobilized N compounds in the soil. Materials removed by 0.1 M $Na_4P_2O_7$ were associated with polyvalent cations in the soil. Materials such as cytoplasmic constituents, released from the biomass during ultrasonic vibration or as lytic products would be expected to be adsorbed to inorganic colloids. They should be concentrated in the < 0.04 μm-size fraction. Cell wall and other particulate debris with a faster setting velocity would be expected to appear in larger-sized sedimentation fractions.

On a incorporé à deux sols en cylindres au champ du ^{15}N qu'on a ensuite laissé s'équilibrer pendant deux saisons de culture. Les sols marqués ont été fractionnés pour établir l'effet des colloïdes organiques et inorganiques sur la stabilisation du ^{15}N fixé. Les matières organiques extraites au NaOH 0.5 N, sans prétraitement, contenaient plus de ^{15}N que celles extraites par le même réactif après décalcification et enlèvement des sesquioxydes au dithionite et par HCl. Les deux types d'extraits avaient la même composition en acides aminés et des degrés semblables d'hydrolysabilité. Un système de fractionnement utilisant une première extraction au NaOH 0.1 M–$Na_4P_2O_7$ 0.1 M, suivie de traitement aux ultrasons et de peptisation dans H_2O a donné une fraction d'acide humique et une fraction de sédiments (< 0.04 μm) différant fortement par le degré d'hydrolysabilité, la teneur en ^{15}N et en N acido-aminé. Le N associé aux colloïdes inorganiques < 0.04 μm et le N restant en

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solution après retrait des grosses particules constituait 50% du N acido-aminé dans le sol argileux et 40% en loam sableux fin. L'enlèvement des sesquioxydes suivi d'une seconde extraction par NaOH 0.5 N a abaissé la teneur en N des fractions colloïdales des deux sols, ce qui montre que les composés amorphes de Fe et de Al à la surface des particules d'argile sont probablement les agents qui lient le N organique aux colloïdes inorganiques. Les auteurs émettent l'hypothèse que la technique de fractionnement sans hydrolyse laquelle, dans une large mesure, est basée sur la dispersion des colloïdes inorganiques–organiques, et les analyses de sédiments pourraient servir à expliquer la cinétique des composés azotés immobilisés par voie microbienne. Les matières enlevées par $Na_4P_2O_7$ 0.1 M se rattachent aux cations polyvalents du sol. On peut supposer ainsi que des matières comme les composants cytoplasmiques dégagés de la biomasse par vibration ultrasonique ou par voie lytique sont adsorbés sur les colloïdes inorganiques. Ils se concentreraient donc dans les particules de calibre < 0.04 μm. Les déchats des membranes cellulaires et d'autres particules possédant un taux de sédimentation plus rapide se trouveraient, quant à eux, dans les fractions à particules plus grosses.

Approximately 60–80% of the N in most soils is released by hydrolysis, in 6 N HCl. This results in amino acids, amino sugars, NH_4^+, and an unidentified fraction (Bremner 1967). During mineralization, N is released from all fractions containing HCl hydrolyzable-N, but the amino acid portion comprising 20–50% of soil organic N is quantitatively the most important (Almieda et al. 1969; Bremner 1967; Moore and Russell 1968, 1970; Reid et al. 1969; Sowden 1968; Stewart et al. 1963). Hydrolytic techniques therefore have indicated that the amino acid fraction is important in supplying N to the mineral pool through mineralization. They also are useful for identifying the decomposable carbon (Martel and Paul 1974). This drastic technique, however, cannot differentiate between cellular and noncellular materials and does little to define the effect of organic and inorganic colloids on stabilization of organic N.

The extraction-characterization method outlined by Anderson et al. (1974) represents a departure from the classical concept of removing as much of the organic material as possible from the mineral portion before studying the organic fraction. Fractionation of clay and its associated organic material as a unit, followed by hydrolysis and measurements of defined hydrolytic products, would seem to be a logical approach to the study of the dynamics of soil organic components. In this way soil can be treated as an entity. The present study examines the removal of the organic matter associated with sesquioxides, and dispersion of the clay–organic matter complex before hydrolysis to determine if it provides useful information on the disposition of recently immobilized N. The fractionation of soil-N and ^{15}N-N was also compared using the phenol extraction system of Biederbeck and Paul (1973).

MATERIALS AND METHODS

Two Orthic Dark Brown Chernozemic soils were used. The Bradwell soil is a fine sandy loam developed on a medium to moderately fine textured glacio-lacustrine deposit and contains 0.21% N, 1.83% organic C and has a surface *p*H of 6.7. The Sutherland clay loam soil has a surface *p*H of 7.4, contains 0.26% N, 2.36% organic C and is developed on variable clayey glacio-lacustrine deposits (Ellis et al. 1970).

Labelling of Soil

Ammonium nitrate-N (6.132 atom percent excess ^{15}N, both NH_4-N and NO_3-N labelled) was added, in the spring of 1967, to the top 10 cm of soil in 30-cm diam steel cylinders placed in the soil to a depth of 90 cm (Myers and Paul 1971). Sufficient N was added to supply 112 kg N/ha. Two crops of wheat were grown (1967 and 1968) after which the top 15 cm of soil was removed from the cylinders, air-dried and stored. Portions (200 g) of this stored soil were removed and ground to pass a 60-mesh sieve. Subsamples were then used for the fractionation studies reported herein.

Fractionation Procedures

(1) The mobile/nonmobile extraction followed by ultrasonic vibration is illustrated in Fig. 1.
(2) The NaOH-$Na_4P_2O_7$ extraction followed by ultrasonic vibration was used as reported by Anderson et al. (1974). Material in suspension or solution after all particles < 0.04 μm had been spun down was designated fraction B. Particle size separations were made by centrifugation and decantation.
(3) The phenol extraction of humic substances was used as described by Biederbeck and Paul (1973).

Analytical Techniques

(1) TOTAL N was determined using a semimicro-Kjeldahl digestion followed by steam distillation (Bremner 1965a,c) and titration to *p*H 4.8 using standard H_2SO_4 and an automatic titration system.
(2) AMINO ACID-N was determined using the ninhydrin–ammonia method described by Bremner (1967) after 6 N HCl hydrolysis under reflux for 16 h.
(3) ^{15}N ANALYSES. Samples were analyzed according to the procedure outlined by Johns (1971). Analyses were performed on a MAT GD

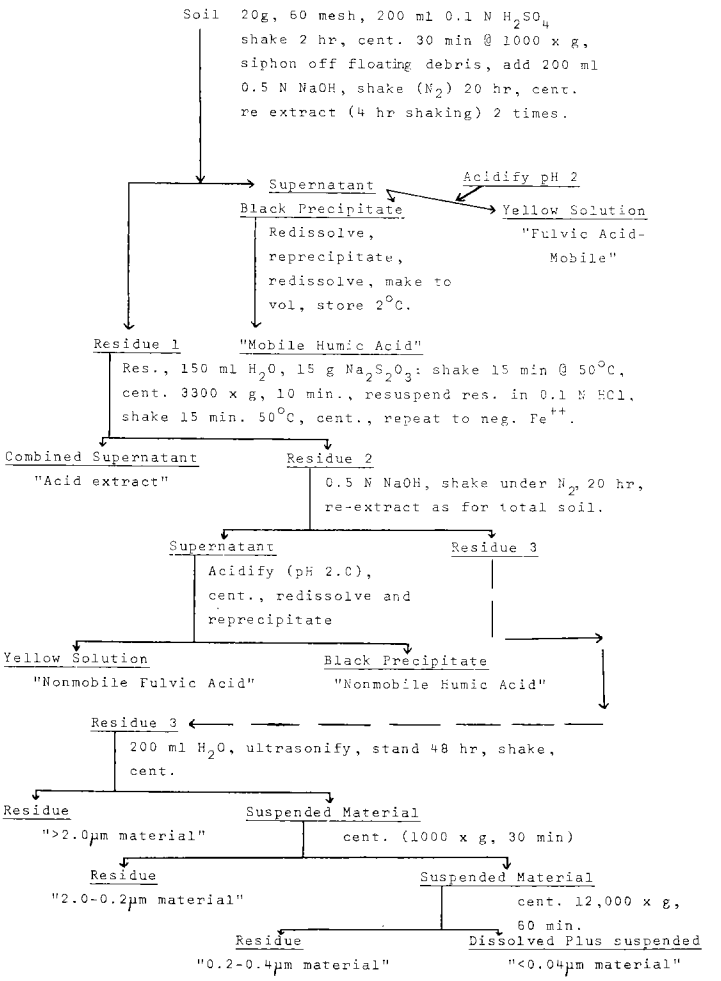

Fig. 1. Extraction of soil organic matter with NaOH-$Na_2S_2O_3$-HCl and sonication.

150 mass spectrometer. N evolution from NH_4^+ was performed on the mass spectrometer using sodium hypobromite oxidation.

RESULTS

Nitrogen Contents of Various Fractions

(1) MATERIAL FLOATED OFF AND REMOVED IN ACID EXTRACT. In the Sutherland (clay loam) and Bradwell (fine sandy loam) soils, respectively, 5 and 4% of the total soil-N were removed in the initial flotation and discarded. Labelled-N contained in this fraction totalled 2.7 and 6.2%, indicating more undecomposed residue in the sandy (Bradwell) soil after 2 yr of field incubation.

The dithionite-HCl extract contained 2.8% of the soil-N and 7.2% of the labelled-N in the Sutherland soil. In the Bradwell soil, 4.6% of the soil-N and 8.6% of the labelled-N were contained in this fraction, indicating a greater proportion of the labelled-N than soil-N in the acid extract.

(2) N CONTENT OF VARIOUS HUMIC AND FULVIC FRACTIONS. The main differences between fractions obtained from the Bradwell and Sutherland soils when extracted with the $NaOH/Na_2S_2O_4$, HCl/NaOH system was in the relative N contents of the mobile and nonmobile humic acid (HA) fraction (Table 1). Mobile material is defined as organic matter extracted with 0.5 N NaOH prior to decalcification and removal of sesquioxides. Nonmobile organic matter is defined as that soluble in 0.5 N NaOH after decalcification and removal of sesquioxides. The nonmobile HA of the clay (Sutherland) soil contained 16% of the soil-N, substantially more than the mobile HA (9%). With the Bradwell soil, the reverse held and the mobile HA fraction (obtained by NaOH extraction alone) contained 19% of the soil-N, whereas the nonmobile fraction contained 12%. Labelled-N in the Bradwell soil exhibited a similar trend with 20% in the mobile HA and 8% in the nonmobile. In the Sutherland soil, however, labelled-N was evenly distributed between the two fractions. Much of the recently added N was removed by the initial NaOH extraction. Hence the mobile organic materials of both soils were much more highly labelled than the nonmobile materials (Table 1).

Table 1. Nitrogen contained in fractions of Sutherland and Bradwell soils obtained by the $NaOH/Na_2S_2O_4$, HCl/NaOH fractionation system

	Sutherland*			Bradwell**		
Fraction	Soil-N (μg/g)	Labelled-N (μg/g)	L/S†	Soil-N (μg/g)	Labelled-N (μg/g)	L/S†
Mobile HA	224	1.9	8.4	400	4.0	10.0
Nonmobile HA	409	1.8	4.4	242	1.6	6.6
Mobile FA	550	3.5	6.3	491	5.6	11.4
Nonmobile FA	166	0.2	1.2	97	0.8	8.2
Sedimentation fractions						
<0.04 μ	436	1.3	2.9	324	1.8	5.6
0.04–0.20 μ	215	0.5	2.3	74	0.4	5.4
0.20–2.0 μ	188	0.3	1.5	127	0.9	7.1
>2.0 μ	208	0.5	2.4	158	0.9	5.7
Total (μg/g)	2,396	10.0		1,913	16.0	
	*μgN/g	**μgN/g				
Total soil-N	2,598	2,088				
Labelled-N	11.1	19.5				

†L/S = degree of labelling = mg ^{15}N/g soil-N.

The $NaOH\text{-}Na_4P_2O_7$ extraction system did not produce any major differences in the relative distribution of HA-A and FA-A between the two soils (Table 2). Fulvic acid A was the most highly labelled fraction obtained and contained larger quantities of labelled-N than the humic acid fraction.

(3) SEDIMENTATION FRACTIONS. Sedimentation fractions, obtained after $NaOH/Na_2S_2O_4$, HCl/NaOH extraction contained less N (Sutherland, 40%; Bradwell, 33%) than after $NaOH\text{-}Na_4P_2O_7$ extraction (Sutherland, 63%; Bradwell, 55%). The $NaOH\text{-}Na_4P_2O_7$ mixture thus extracted less of the soil- and labelled-N associated with inorganic particles, principally those of colloidal size. The clay–humus fraction (<0.04 μm), after extraction with the $NaOH/Na_2S_2O_4$, HCl/NaOH system, contained 18% of the Sutherland soil-N and 13% of the labelled-N. Similar quantities (17% soil-N; 11% labelled-N) were present in Bradwell soil (Table 1). The analogous clay–humus fraction (fraction B) produced by the $NaOH\text{-}Na_4P_2O_7$/sonication system contained more N (average of 31% for soil-N; 33% for labelled-N) than any other fraction from either extraction system.

Hydrolyzable N of the Fractions

The mobile and nonmobile HA within each soil were equally hydrolyzable, although those from the Bradwell soil were less hydrolyzable than those from the Sutherland. With the $NaOH\text{-}Na_4P_2O_7$/sonication system, N in HA-A was less hydrolyzable than in other fractions of both soils (Table 3). Hydrolyzability of soil-N in the 0.20-0.04-μm (fine clay) size fraction of both soils obtained by both techniques was greater than that of labelled N. This fraction may contain a quantity of fixed NH_4^+ released on hydrolysis (Freney and Miller 1970).

Labelled-N in fraction B from the Sutherland soil was more hydrolyzable than from the Bradwell soil; otherwise, no differences were observed between soils in the degree of hydrolyzability of labelled-N in the various fractions. Except for the previously noted fine clay fractions, labelled-N was generally much more hydrolyzable than nonlabelled or native soil-N, demonstrating that recently immobilized N has not uniformly entered the soil organic matter pool.

Amino Acid-N in the Fractions

Results reported in the literature indicate

Table 2. Nitrogen contained in fractions of Sutherland and Bradwell soils obtained by the $NaOH\text{-}Na_4P_2O_7$ fractionation system

	Sutherland*			Bradwell**		
Fraction	Soil-N (μg/g)	Labelled-N (μg/g)	L/S†	Soil-N (μg/g)	Labelled-N (μg/g)	L/S†
HA-A	438	1.9	4.3	495	3.4	6.9
FA-A	401	2.2	5.5	375	4.6	12.6
Sedimentation fractions						
Fr. B						
<0.04 μm	888	4.4	5.0	591	5.0	8.5
0.04–0.20 μm	128	0.3	2.3	92	0.6	6.5
0.20–2.0 μm	323	1.0	3.1	211	2.0	9.5
>2.0 μm	289	1.0	3.5	254	2.8	11.0
Total (μg/g)	2,467	10.8		2,018	18.5	
	*μgN/g	**μgN/g				
Total soil-N	2,598	2,088				
Labelled-N	11.1	19.5				

†L/S = degree of labelling = mg ^{15}N/g soil-N.

Table 3. Degree of hydrolyzability of N in fractions of Sutherland and Bradwell soils

Fraction	Hydrolyzable N as % of total-N in fraction			
	Sutherland		Bradwell	
	Soil	Labelled	Soil	Labelled
$NaOH/Na_2S_2O_4$, HCl/NaOH system				
Mobile HA	80	88	70	90
Nonmobile HA	71	89	68	88
<0.04 μm	79	85	92	97
0.04–0.20 μm	99	89	100	88
0.20–2.0 μm	81	100	91	100
>2.0 μm	84	94	71	90
$NaOH$, $Na_4P_2O_7$ ultrasonication system				
HA-A	67	85	59	86
Fr. B				
<0.04 μm	92	94	88	88
0.04–0.20 μm	100	97	96	100
0.20–2.0 μm	84	93	82	97
>2.0 μm	88	98	88	100

that a large portion of the immobilized mineral-N is converted into amino acid-N (Stewart et al. 1963). This fraction supplies a large quantity of N to the mineral-N pool (Stewart et al. 1963). The amino acid-N content of all fractions isolated was therefore measured. The most highly labelled amino acids isolated by the NaOH/$Na_2S_2O_4$, HCl/NaOH system were in the humic acids and mobile fulvic acid (Table 4). Amino acids formed a larger portion of the N hydrolyzed from the humic acids than from other fractions produced by this fractionation technique, although this system did not appear to concentrate amino acid-N in any individual fraction.

Fraction B (< 0.04 μm) of the NaOH-$Na_4P_2O_7$ system contained 50 and 40% of the labelled amino acid-N in the Sutherland and Bradwell soils, respectively (Table 5). This was the largest single amino acid-N fraction isolated by either system. The

Table 4. Amino acid-N content of fractions of Sutherland and Bradwell soils obtained by the NaOH/$Na_2S_2O_4$, HCl/NaOH fractionation system

Fraction	Soil-N (μg/g)	Labelled-N (μg/g)	L/S‡	% of labelled AA-N†	AA-N as % of hyd.-N in fraction	
					Soil	Labelled
Sutherland						
Mobile HA	95	0.99	10.4	25	53	59
Nonmobile HA	138	0.94	6.8	24	43	61
Mobile FA	126	0.98	7.8	25	23	27
Nonmobile FA	40	0.03	0.8	1	24	15
<0.04 μm	155	0.48	3.1	12	45	47
0.04–0.20 μm	75	0.17	2.3	4	35	38
0.20–2.0 μm	65	0.15	2.3	4	37	50
>2.0 μm	77	0.21	2.7	5	44	45
Bradwell						
Mobile HA	124	2.12	17.1	37	44	59
Nonmobile HA	76	0.82	10.8	14	46	58
Mobile FA	111	1.16	10.5	20	23	21
Nonmobile FA	23	0.17	7.4	3	24	21
<0.04 μm	96	0.74	7.7	13	32	42
0.04–0.20 μm	14	0.07	5.0	1	19	20
0.20–2.0 μm	36	0.33	9.2	6	31	37
>2.0 μm	39	0.28	7.2	5	35	35

†AA, amino acid.
‡L/S = mg ^{15}N/g soil-N.
= degree of labelling.

$NaOH\text{-}Na_4P_2O_7$ extraction left more amino acid-N associated with inorganic colloids than did the $NaOH/Na_2S_2O_4$, HCl/NaOH system. Humic acid A contained approximately one-half as much labelled amino acid-N as did fraction B. However, the amino acid-N in this fraction was more highly labelled.

Centrifugation of < 0.04 μm Material

When brought to *p*H 2.0, fraction B yielded a grey flocculent precipitate with very little N remaining in solution. The grey color of the precipitate and the cloudy appearance of the material when dissolved (*p*H 7.5) suggested that the N may be associated with colloidal inorganic material. Anderson et al. (1974) reported a much higher ash content in this fraction (which they termed humic acid B) than in other humic acid fractions (humic acid A) obtained from the soils they studied. They also presented evidence for the presence of expanding lattice clays. To determine if the organic material in this fraction was intimately associated with clay and, if so, could fraction B be further subdivided by centrifugation, this fraction was centrifuged at 40,000 × *g* for 60 min. A large portion of the material settled out with this treatment. The degree of labelling of the N, percent hydrolyzability and amino acid-N content of the precipitate was similar to that of the supernatant. Hydrolyzability of labelled-N and soil-N was similar in both portions, but amino acids made up a greater proportion of the labelled-N than soil-N. It was concluded that centrifugation did not produce any fundamental fractionation of < 0.04 μm material.

Phenol Extraction of HA-A

The high degree of labelling of the amino acids in the HA-A fraction suggests that a quantity of proteinaceous N may be adsorbed to the surface of HA-A "core" material. To check this hypothesis, the HA-A from the Sutherland soil was ex-

Table 5. Amino acid-N content of Sutherland and Bradwell soils obtained by the NaOH, $Na_4P_2O_7$ fractionation system

Fraction	Soil-N (μg/g)	Labelled-N (μg/g)	L/S†	% of labelled AA-N‡	AA-N as % of hyd.-N in fraction	
					Soil	Labelled
Sutherland						
HA-A	131	0.91	6.9	20	45	56
FA-A	87	0.58	6.7	13	22	26
Fr. B						
<0.04 μm	377	2.28	6.0	50	46	55
0.04–0.20 μm	44	0.15	3.4	3	34	52
0.20–2.0 μm	114	0.43	3.8	9	42	46
>2.0 μm	108	0.21	1.9	5	42	21
Bradwell						
HA-A	123	1.67	13.6	27	42	57
FA-A	78	0.72	9.2	11	21	16
Fr. B						
<0.04 μm	219	2.51	11.6	40	42	57
0.04–0.20 μm	23	0.18	7.8	3	26	30
0.20–2.0 μm	63	0.80	12.7	13	36	41
>2.0 μm	90	0.22	2.4	5	35	11

†L/S = mg ^{15}N/g soil-N.
= degree of labelling.
‡AA, amino acids.

tracted with 75% phenol solution. This treatment removed 132 μg soil-N (31% of total HA-A-N) and 0.81 μg labelled-N per gram (43% of labelled HA-A-N). The phenol extract contained 5.5 mg ^{15}N/g soil-N (1.48 times as great as the original HA-A). Biederbeck and Paul (1973) used phenol to remove an aliphatic, amino-rich moiety from humic acids. The material removed is generally considered to be adsorbed to the surface by hydrogen bonding. These data indicate that HA-A contains a quantity of highly labelled N held possibly by hydrogen bonding.

DISCUSSION

The large quantity and high degree of labelling of the humic N removed by the mobile/nonmobile fractionation system ($NaOH/Na_2S_2O_4$, HCl/NaOH) was anticipated. The rather severe treatments could be expected to cause considerable redistribution of extracted material (Sauerbeck and Fuhr 1968). The mobile humic fractions (HA and FA) isolated by this technique had similar degrees of labelling. Therefore, either the total humic fraction is the active fraction or a large amount of active N is coextracted with it. The latter appears more probable. A further purification or fractionation is required, thus making it increasingly difficult to relate this type of result back to the soil system.

It would appear preferable to use a milder extraction in which less material is extracted but in which N may be partitioned.

In view of the large quantity of labelled-N found in fraction B (< 0.04 μm after $NaOH$-$Na_4P_2O_7$ extraction and sonication), its high amino acid content and the distinct differences in hydrolyzability between this fraction and HA-A, the $NaOH$-$Na_4P_2O_7$/sonication system is considered superior for a study of N turnover. The behavior of amino acid-N and the effect of colloidal inorganic constituents can be more clearly elucidated using this technique.

The fine clay fraction is sufficiently different from the 0.20- to 2.0-μm and $>$ 2.0-μm fraction to be retained as a separate fraction. All material with an apparent diameter 0.2 μm may be retained as one fraction or combined after $Na_4P_2O_7$ extraction and sonication. This fraction probably contains a large quantity of particulate organic matter (microbial and plant) and material physically adsorbed to sand- and silt-sized particles.

It is suggested that a $NaOH$-$Na_4P_2O_7$/sonication system of fractionating soil would be useful in studies of N turnover through microorganisms in soil. Sonic rupture of microbial cells is used to separate microbial cytoplasm from the cell wall components in aqueous systems (Salton 1964). The components are then separated by centrifugation with cell wall and particulate debris settling more rapidly than membrane fragments and ribosomes. Cytoplasmic material tends to remain in solution. The presence of soil colloids offers a large surface area onto which organic molecules or particles could adsorb. Soluble cytoplasmic material, composed of relatively flexible molecules containing numerous charged sites would be expected to become associated with inorganic (and organic) colloids by various mechanisms as described by Greenland (1971), and by Mortland (1970). Particulate material can be expected to settle out, upon centrifugation, with larger-sized mineral particles. Results of application of the $Na_4P_2O_7$/sonication system to studies of the dynamics of microbially produced C and N in soil (McGill et al. 1975) indicate that particulate components have a slower turnover rate than do components adsorbed onto fine clay before or during sonication.

It is concluded that a large quantity of the N associated with fine inorganic colloids is held through the effect of sesquioxides. The greater amount, and higher N content of the nonmobile HA of the Sutherland clay, and the higher N content of the mobile HA of the sandy Bradwell soil are consistent with this hypothesis. The reduced N contents in the sedimentation fractions, notably in the $>$ 0.04-μm fraction, after dithionite–HCl

treatment is further evidence. This indicates that the large, amino-rich moiety (fraction B) is probably stabilized by sesquioxides rather than by entrapment within the clay lattice. This, together with the high degree of hydrolyzability of N in high clay soils (Bremner 1965a,b,c) indicates that clay minerals, or amorphous coatings on them, may adsorb aliphatic biologically labile N, inhibiting humification. High fertility status of many heavy-textured soils may result from microaggregate disruption followed by microbial attack on organic-N thus made accessible. N associated with inorganic colloids is readily mineralized by microorganisms (Chichester 1970). Results reported herein are in agreement with those of Kyuma et al. (1969) who demonstrated the high hydrolyzability of organic-N associated with inorganic colloids and its high amino acid-N content.

Material extracted by a $NaOH\text{-}Na_4P_2O_7$ mixture is more recalcitrant (lower hydrolyzability) than other fractions and evidence obtained by phenol extraction of HA-A suggests that it consists of a highly condensed aromatic core surrounded by adsorbed amino-rich, aliphatic constituents. This conclusion is further supported by data of Anderson et al. (1974) which indicate that HA-A is more highly humified and aromatic than fraction B. This is similar to the general concept of HA as outlined by Haworth (1971). This fraction does not appear to be intimately associated with soil clay minerals, but exists associated with various cations in soil. Although adsorption of recently immobilized N onto both organic and inorganic colloids is evident, more amino acid-N is stabilized by association with clay minerals than with humic components in the two soils studied.

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