NITROGEN TRANSFORMATIONS DURING THE BIOLOGICAL DECOMPOSITION OF STRAW COMPOSTED WITH INORGANIC NITROGEN

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(With Two Text-figures)

Although little is yet known about the decomposition of plant residues in the soil, considerable information is available regarding the chemical changes that accompany the rotting or composting of plant materials in the absence of soil. The biological decomposition of cereal straws has received particular attention and its general course is now well established. The main feature of aerobic decomposition in the presence of a supply of available nitrogen, which is necessary for speedy rotting, is the rapid decomposition of the carbohydrate material, especially cellulose, accompanied by an increase in the amount of organically-combined nitrogen. Hutchinson & Richards (1921) were the first to recognize the importance of an adequate supply of available nitrogen in decomposition. They determined the amount of nitrogen necessary for rapid decomposition of various plant materials and introduced the term 'nitrogen factor' which may be defined as the number of grams of additional inorganic nitrogen immobilized as organic nitrogen by 100 g. of any plant material in the process of decomposition. The 'nitrogen factor' of common straws is generally about 0·8. Besides a supply of available nitrogen, the other factors found necessary to secure rapid decomposition of straw were a moisture content of about 75 %, an adequate air supply, and an alkaline or neutral reaction. Ammonium carbonate was found to be the most satisfactory form of added nitrogen; ammonium sulphate rapidly developed an acid reaction which checked decomposition, and considerable loss of nitrogen was observed when sodium nitrate was used.

It is generally stated that the immobilization of inorganic nitrogen during the composting of straw and other plant materials is due to its conversion to microbial protein. However, no evidence to support this assumption has been reported in the literature and few attempts have been made to estimate the amounts of protein or other forms of organic nitrogen synthesized during composting. Most workers have followed Waksman & Gerretsen (1931) who used the method of proximate analysis devised by Waksman & Stevens (1930) to study changes in the organically-combined nitrogen during the composting of oat straw with ammonium phosphate and estimated protein from the amount of water-insoluble nitrogen using the conventional factor of 6·25.

The object of the work reported in this paper was to obtain more precise information regarding the chemical nature of the nitrogenous organic complexes synthesized during the composting of straw with inorganic nitrogen. It was hoped that such information might help to account for the fact that the organic nitrogen compounds in composts are very resistant to decomposition in soil. A further object of the work was to discover if there are any similarities in nitrogen distribution and amino-acid composition between the nitrogenous organic complexes in soils and composts.

While this work was in progress Mattingly (1952) published a preliminary account of an investigation of the changes in the distribution of nitrogen during the decomposition of straw composted with sewage sludge in which reference was made to a study of nitrogen transformations during the composting of straw with inorganic nitrogen. Communication with Dr Mattingly revealed that he had followed nitrogen changes during the decomposition of wheat straw composted with ammonium sulphate and that a paper giving the results of this work was being prepared for publication. Reference to this paper (Mattingly, 1954) will show that where the investigations overlap Dr Mattingly's findings are in agreement with those of the present work.

MATERIALS, METHODS AND RESULTS

Air-dried chaffed samples of mature oat and wheat straw were used. Their nitrogen contents (dry matter basis) were oat straw 0·75 %, wheat straw 0·65 %. The conditions adopted for composting the straws were those found by previous workers to effect rapid decomposition, ammonium carbonate being used as source of nitrogen. As the 'nitrogen factor' of straw is generally about 0·8, nitrogen was

supplied at the rate of approx. 1 g. per 100 g. of straw to ensure that there would be a small excess of available nitrogen over the amount required for decomposition.

The method adopted in the oat straw decomposition experiments was as follows. 50 g. samples of the air-dried straw were transferred to tared wide-necked 1000 ml. conical flasks and moistened as uniformly as possible with 150 ml. of a solution containing 0·508 g. of nitrogen as ammonium carbonate. The flasks were then stoppered and incubated at 30° C. for periods of 10, 20, 30, 60 and 100 days. At 2-day intervals during incubation the flasks were shaken thoroughly to promote even distribution of moisture and aerated by hand-bellows. At the end of each incubation period a flask was removed from the incubator, weighed, and after thorough mixing of its contents, samples were taken for dry matter, total-N, ammonia-N and nitrate-N determinations. The remainder of the sample was hydrolysed with acid and analysed by the methods described below.

A different procedure was used in the wheat straw decomposition experiment because a large sample of the rotted material was required for other investigations. In this experiment 1 kg. of the air-dried straw was spread in a thin layer over the bottom of a large glass tank and moistened uniformly with 3 l. of the ammonium carbonate solution used in the oat straw decomposition experiments. The tank was then covered with a glass plate and decomposition was allowed to proceed at room temperature, the contents of the tank being mixed and aerated at 4-day intervals. After a period of 120 days the contents of the tank were weighed, mixed thoroughly and sampled as in the oat straw experiments.

Extensive fungal growth was observed during the initial, but not in later stages of the oat straw decomposition. Fungal development was also noted in the early stages of the wheat straw decomposition but it was much less extensive.

Analysis of samples

Dry matter was determined by drying weighed samples overnight at 105° C.

Total-N was determined by the Kjeldahl method using K_2SO_4 and $CuSO_4$, the salicylic acid-thiosulphate modification being used for samples containing nitrate-N.

Ammonia- and nitrate-N were extracted with N-K_2SO_4 made 0·1 N with respect to H_2SO_4. Ammonia-N in the extract was estimated by distillation with MgO at room temperature in Conway (1947) microdiffusion units and Nesslerization of the distillate (Bremner & Shaw, 1954). Nitrate-N was estimated colorimetrically with brucine after clarification of the extract with activated charcoal, the method adopted being essentially that described by Peech & English (1944).

The amounts of dry matter, total-, organic-, ammonia- and nitrate-N in the straw composts after various periods of time are given in Table 1. Small amounts of ammonia- and nitrate-N were detected in both of the straws used for preparation of the composts. No nitrite-N could be detected in any of the samples analysed.

Table 1. *Nitrogen and dry matter changes in straw-ammonium carbonate composts*

(Oat straw rotted at 30° C., wheat straw at room temperature; moisture contents of composts originally 79 %)

Period of decomposition (days)	Dry matter (g.)	N present (g.)				Nitrogen factor	N in dry matter (%)				Loss of N (%)
		Total	NH_4	NO_3	Organic		Total	NH_4	NO_3	Organic	
Oat straw											
0	100·0	1·87	1·12	< 0·01	0·75	—	1·87	1·12	< 0·01	0·75	—
10	87·1	1·81	0·52	0	1·29	0·54	2·08	0·60	0	1·48	3·2
20	75·7	1·69	0·25	0	1·44	0·69	2·23	0·33	0	1·90	9·6
30	65·5	1·65	0·04	0	1·61	0·86	2·52	0·06	0	2·46	11·7
60	56·2	1·63	0·03	0	1·60	0·85	2·90	0·05	0	2·85	12·8
100	41·0	1·62	0·02	< 0·01	1·60	0·85	3·95	0·05	< 0·01	3·90	13·4
Wheat straw											
0	100·0	1·75	1·10	< 0·01	0·65	—	1·75	1·10	< 0·01	0·65	—
120	40·6	1·61	0·06	0·23	1·32	0·67	3·97	0·15	0·57	3·25	8·0

Hydrolysis of samples

Two methods of acid hydrolysis were employed. Method 1 was used for hydrolysis of protein material present, the amino-acids liberated being subsequently estimated and identified. Method 2 was used to liberate amino sugars from hexosamine complexes (Smithies, 1952; Bremner & Shaw, 1954) and for estimation of 'amide nitrogen' (Shore, Wilson & Stueck, 1935).

Method 1. The sample was boiled with 6 N-HCl (20 ml./g. of dry matter) under reflux for 18 hr., a few drops of capryl alcohol being added to prevent foaming. The hydrolysis mixture was then filtered, the residue washed thoroughly with water, and the combined filtrate and washings concentrated several

times *in vacuo* to remove HCl. The residue was taken up with water, the solution made to volume, and aliquots were taken for total-, ammonia-, volatile base- and α-amino-N determinations. The remainder of the solution was concentrated to small volume *in vacuo*, desalted by the method of Consden, Gordon & Martin (1947), and examined by paper partition chromatography.

Method 2. The sample was heated with 6 N-HCl (20 ml./g. of dry matter) under reflux for 6 hr. at 100° C. After filtration and removal of HCl as described above aliquots of the hydrolysates were taken for total-, ammonia- and amino sugar-N estimations and for paper chromatographic examination.

It was found that when the rotted wheat straw was subjected to acid hydrolysis a considerable proportion of the nitrate present was reduced and appeared as ammonia in the hydrolysate. This sample was therefore leached with 0·2 N-H_2SO_4 to remove the nitrate present, washed with water and dried and analysed for total-N before hydrolysis. As the other samples examined did not contain significant amounts of nitrate-N they did not require this pre-treatment before hydrolysis.

Analysis of the hydrolysates

Total-N was determined by a micro-Kjeldahl method using $SeO_2 : CuSO_4.5H_2O : K_2SO_4$ 1 : 1 : 8 as catalyst.

Volatile base-N was estimated by distillation *in vacuo* at 40° C. with MgO into N/70-HCl and back titration of the excess acid with N/70-NaOH, methyl red masked by methylene blue being used as indicator. Distillations were conducted in an all-glass apparatus similar to that described by Pucher, Vickery and Leavenworth (1935).

Ammonia-N was estimated by Nesslerizing volatile base distillates. The tints obtained appeared to be identical with those given by ammonia. Ammonia-N in the hydrolysates was also estimated by the microdiffusion method described by Bremner & Shaw (1954). The results obtained by these two methods were in close agreement.

α-Amino-N (amino-acid-N) was determined by the manometric ninhydrin method of Van Slyke, Dillon, MacFadyen & Hamilton (1941).

Amino sugar-N was estimated by the Elson & Morgan (1933) colorimetric method modified according to Blix (1948) and Immers & Vasseur (1950) and by the alkaline decomposition method of Tracey (1952). The results obtained when the amino sugar-N contents of the rotted straw hydrolysates were estimated by the alkaline decomposition method were 5–10 % higher than those obtained using the colorimetric method. The figures given in Table 2 represent the means of the results given by the two methods.

Table 2. *Distribution of nitrogen liberated by acid hydrolysis of organic nitrogen compounds present in straw-ammonium carbonate composts at different stages of decomposition*

(Acid-soluble-, ammonia-, volatile base- and α-amino-N estimated after hydrolysis with 6 N-HCl at 110° C. for 18 hr. Amino sugar-N and 'amide-N' estimated after hydrolysis with 6 N-HCl at 100° C. for 6 hr. Results expressed as percentages of the organic nitrogen in the composts.)

Period of decomposition (days)	Acid-soluble-N	Ammonia-N	Volatile base-N	α-Amino-N	'Amide-N'	Amino sugar-N
Oat straw						
0	82·1	10·8	10·9	51·6	8·2	0
10	81·3	12·7	—	49·1	—	—
20	80·1	10·4	10·4	47·6	—	—
30	82·0	11·7	—	48·1	—	—
60	81·2	12·6	—	47·8	8·7	3·6
100	80·1	13·0	13·0	48·2	8·9	3·4
Wheat straw						
0	82·5	11·3	11·4	51·0	8·8	0
120	68·1	13·8	13·9	38·6	10·4	4·0

The amounts of acid-soluble-, ammonia-, volatile base-, α-amino-, 'amide-' and amino sugar-N liberated by hydrolysis of the nitrogenous organic complexes in the composts at different stages of decomposition are given in Table 2. 'Amide-N' has been estimated from the ammonia-N liberated by hydrolysis with 6 N-HCl at 100° C. for 6 hr. Volatile base is assumed to represent ammonia and substituted ammonia.

Nitrogen changes during the composting of oat straw with ammonium carbonate are illustrated graphically in Fig. 1.

Paper chromatography

The amino acids liberated by hydrolysis of the composts with 6 N-HCl for 18 hr. were identified by two-dimensional paper chromatography, the technique used being essentially that described by Dent (1948). Chromatograms were run in one direction with phenol in an atmosphere of NH_3 and HCN,

and in the other with 'collidine' in an atmosphere of diethylamine. The 'collidine' used was a mixture of equal parts of 2 : 4 : 6-collidine and the 2 : 4/2 : 5-lutidine supplied by Light & Co., the bases being redistilled before use. Whatman No. 4 filter paper was used throughout. After drying, the chromatograms were sprayed with ninhydrin (0·1 %, w/v) in $CHCl_3$ and the strengths of the spots detected were estimated visually from their size and colour intensity using an arbitrary scale of 10 units (Table 3). Proline and hydroxyproline, which give yellow and orange ninhydrin colours respectively, were assessed as strong (S), medium (M) and weak (W). The limitations of this method of spot strength estimation have already been discussed by Crumpler, Dent & Lindan (1950), but it may be emphasized here that the numerical values assigned to colour strengths of individual amino-acid spots are not directly proportional to the amounts present on the chromatogram. Nevertheless, the method does reveal gross differences between the relative amounts of any one amino-acid on different chromatograms and is very useful for purposes of comparison.

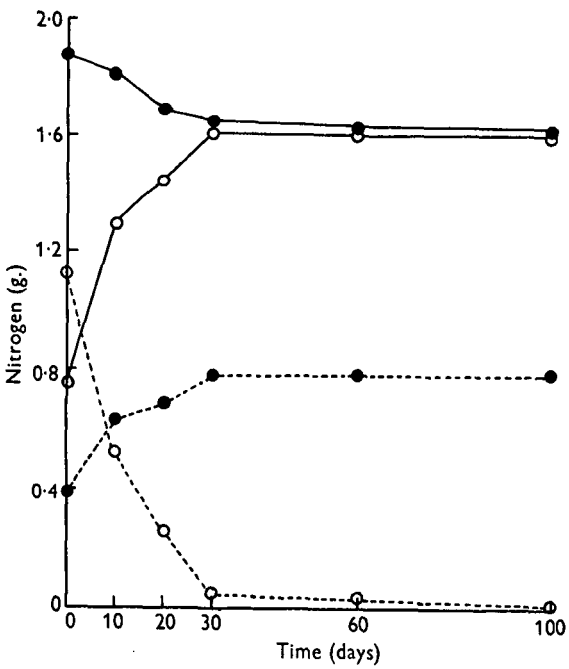

Fig. 1. Changes with time in the amounts of total-, ammonia- and organic-N in oat straw-ammonium carbonate compost made from 100 g. straw (dry matter) and changes in the amounts of α-amino-N liberated by acid hydrolysis of the compost. ●—●, total-N; O—O, organic-N; ●- -●, α-amino-N; O- -O, ammonia-N.

Chromatograms obtained from acid hydrolysates of oat straw and rotted oat straw are reproduced diagrammatically in Fig. 2. These chromatograms and those described in Table 3 were obtained from desalted hydrolysates. Chromatograms obtained from undesalted hydrolysates showed extensive streaking and tailing of the spots.

Cystine and methionine were detected by the ammonium molybdate-H_2O_2 technique of Dent (1948). Histidine, which gives a weak reaction with ninhydrin, was only barely visible on some of the two-dimensional chromatograms, but its presence in all of the hydrolysates examined was readily confirmed by running one-dimensional chromatograms in butanol-acetic acid and spraying with the Pauly reagents (Bremner, 1950).

The substance occupying spot 19 (Fig. 2) appeared to be an α-amino-acid as it could not be detected on chromatograms dusted with $CuCO_3$ (Crumpler & Dent, 1949). Its position was unaffected by H_2O_2 indicating that it was not a sulphur-containing amino-acid (Dent, 1948). Its provisional identification as α, ε-diaminopimelic acid (Table 3) is based on the finding that its position on the two-dimensional phenol-'collidine' chromatograms was identical with that taken up by an authentic sample of α, ε-diaminopimelic acid added as a marker.

The presence of amino sugars in hydrolysates of the rotted straws (Table 2) was confirmed by the finding that two-dimensional chromatograms of these hydrolysates contained a spot (no. 20 in Fig. 2) in the position taken up by glucosamine added as a marker. Further evidence for the presence of amino sugars in the rotted straw hydrolysates was provided by the detection of cherry-red spots identical in R_F value to those obtained with glucosamine or galactosamine when one-dimensional chromatograms of hydrolysates obtained by Method 2 were run in butanol-acetic acid and sprayed with the Elson & Morgan (1933) hexosamine reagents (Partridge, 1948). As glucosamine and galactosamine cannot be distinguished on chromatograms run in these solvents an attempt was made to establish the identity of the amino sugar material present by running chromatograms in solvents reported to effect a separation of the two hexosamines, namely *s*-collidine and butanol-NH_3 (Aminoff & Morgan, 1948). However, the results did not permit any conclusion regarding the identity of the amino sugar material as tests showed that substances present in the hydrolysates prevented a clear separation of added glucosamine and galactosamine. It may be noted that the strength of the amino sugar spot detected on the phenol-'collidine' chromatograms (Table 3) gives no indication of the amount of amino sugar present in the sample chromatographed, as both glucosamine and galactosamine are extensively decomposed on chromatograms run in these solvents (Aminoff & Morgan, 1951).

DISCUSSION

It can be seen from Table 1 and Fig. 1 that the amount of organic nitrogen synthesized by micro-organisms during the oat straw decomposition was maximal at 30 days and did not alter appreciably in the following 70 days. However, as a result of further decomposition and loss of dry matter in this 70-day period the percentage of organic nitrogen in the dry matter of the compost increased from 2·46 to 3·90%. No ammonification of the synthesized organic nitrogen was observed and only trace of the nitrogen synthesized was in the form of protein and that a smaller fraction was in the form of amino sugar.

Interpretation of the results obtained in the wheat straw decomposition experiment is complicated by the fact that the compost was not sampled before the onset of nitrification of the synthesized organic nitrogen. Nevertheless, it is clear from Tables 1 and 2 that the wheat straw decomposition was also accompanied by synthesis of organic nitrogen in the form of protein and amino sugar. The fact that the percentage of α-amino-

Table 3. *Strengths of spots observed on two-dimensional chromatograms of acid hydrolysates of straw composts*

(Strengths of spots are on an arbitrary scale of 10 units. 1 = weak, 10 = strong. Proline and hydroxyproline strengths are indicated by S = strong, M = medium, W = weak; a dash (—) indicates that the substance was not detected. The numbers before each substance refer to the spot numbers in Fig. 2.)

Substances detected	Period of decomposition (days) . . .	Oat straw			Wheat straw	
		0	60	100	0	120
1. Phenylalanine		2	2	3	3	2
2. Leucine, etc.*		10	10	10	10	10
3. Valine		8	9	8	8	8
4. Alanine		8	8	8	9	9
5. Glycine		8	9	9	9	10
6. Threonine		2	2	3	3	3
7. Serine		5	6	6	6	6
8. Glutamic acid		9	9	8	9	9
9. Aspartic acid		8	8	9	8	8
10. Lysine		4	4	4	4	4
11. Arginine		2	2	3	2	3
12. Histidine		<1	1	1	<1	1
13. Tyrosine		2	2	3	2	2
14. β-Alanine		—	1	1	—	1
15. Proline		S	S	M	S	M
16. Hydroxyproline		W?	—	—	W?	—
17. Cysteic acid†		1	<1	<1	1	<1
18. Sulphoxide of methionine		1	1	1	1	1
19. α, ε-Diaminopimelic acid‡		—	1	1	—	1
20. Amino sugar§		—	1	1	—	2

* Leucine, isoleucine or methionine, or mixtures of these compounds.

† Detected on chromatograms of samples of hydrolysate previously treated with H_2O_2 and ammonium molybdate to oxidise cystine. Methionine sulphone, produced by oxidation of methionine, was also detected on these chromatograms.

‡ Provisional identification.

§ Glucosamine, galactosamine or a mixture of these compounds.

amounts of nitrate were detected after 100 days. Comparison of the results given in Table 2 shows that the synthesis of organic nitrogen during the oat straw decomposition did not lead to any marked change in the distribution of the forms of organic nitrogen. Apart from a slight increase in the proportion of 'amide-N' and a slight decrease in the proportion of α-amino-N, the only change in nitrogen distribution found to accompany decomposition was that due to synthesis of amino sugars. The nature of the organic nitrogen synthesized during the oat straw decomposition was not fully established by the methods of analysis employed but it is clear from Table 2 and Fig. 1 that a large fraction nitrogen was considerably smaller in the wheat straw compost than in the oat straw composts (Table 2) suggests that much of the nitrate found in the wheat straw compost was produced by nitrification of protein material synthesized during decomposition.

It is difficult to decide what proportion of the ammonia liberated by acid hydrolysis of the composts was derived from the amide groups of protein material. The method used for estimation of amide nitrogen was essentially that recommended by Shore *et al.* (1935), but the results are of value only for purposes of comparison as the method was never intended for use with materials containing

significant amounts of non-protein nitrogen. Some of the ammonia found in the hydrolysates of the composts was undoubtedly formed by reactions involving the destruction of amino acids and the decomposition of non-protein material. The finding that the amount of ammonia liberated by hydrolysis of the composts with 6 N-hydrochloric acid for 18 hr. was appreciably greater than the amount obtained by hydrolysis for 6 hr. (Table 2) is proof that such reactions do occur. Some of the ammonia found in the hydrolysates of the composts containing amino sugars was certainly formed by decomposition of the hexosamines during acid hydrolysis (see Bremner & Shaw, 1954) and the slight increase in the proportion of 'amide-N' found to accompany straw decomposition (Table 2) may be partly explained by the fact that amino sugar complexes were synthesized during the decomposition of the straw. The fact that amino sugars are unstable towards acid hydrolysis also means that the amounts of amino sugar-N found in the hydrolysates of the mature composts were less than the amounts actually present before hydrolysis. Using the same conditions for hydrolysis, Bremner & Shaw (1954) found that the recovery of amino sugar-N from chitin was about 80 %, and on this basis they estimated the amount of amino sugar-N in various soils by applying a correction factor of 1·25 to the amount of amino sugar-N found after hydrolysis. The results indicated that 5–10 % of the organic nitrogen in the soils examined was in the form of amino sugar. Application of the same correction factor to the results in Table 2 indicates that 4–5 % of the organic nitrogen in the mature composts was in the form of amino sugar.

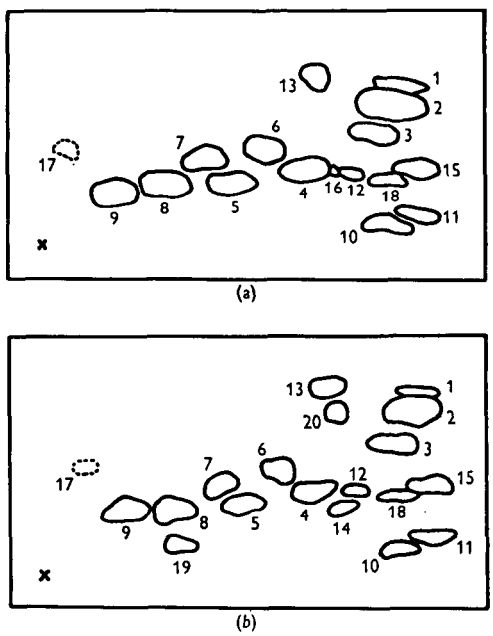

Fig. 2. Diagrams of chromatograms obtained from acid hydrolysates of oat straw (*a*) and oat straw composted with ammonium carbonate for 100 days (*b*). The samples were placed at the bottom left-hand corner of the filter paper. Phenol was run from left to right followed by 'collidine' in an upward direction. The cysteic acid spots (17) were taken from other chromatograms in which the hydrolysates had been previously oxidised with H_2O_2 and ammonium molybdate. A key to the numbers of the spots is given in Table 3.

Charitschkov (1906) and Lugg (1946) have reported that certain amines (e.g. methylamine) react with Nessler's reagent to give colours differing in tint from that obtained with ammonia. The presence of a significant quantity of volatile amine in the hydrolysates of the composts would probably be disclosed therefore by a departure from the normal ammonia tint on Nesslerization of a volatile base distillate or by significant differences between the estimations of volatile base and ammonia. The fact that no such variations were observed (Table 2) indicates that volatile amines could have been present only in traces in the compost hydrolysates.

Comparison of the results given in Table 3 shows that the amino acid composition of rotted straw is not greatly different from that of the unrotted material. The only notable difference detected was that hydrolysates of the rotted straws contained β-alanine and a substance which closely resembled α, ϵ-diaminopimelic acid in its behaviour on paper chromatograms. Both of these substances have been detected in acid hydrolysates of soil (Bremner, 1950).

As Table 1 shows, the loss of nitrogen in the decomposition experiments varied from 3 to 13 %. This loss is considerably less than that noted in similar experiments by other workers. For example, Richards & Shrikhande (1935) found that the loss of nitrogen during the rotting of oat straw with ammonium carbonate at 35° C. for periods up to 56 days varied from 18 to 24 %, and that the loss was greater when ammonium or sodium nitrate was used as source of nitrogen.

Comparison of the results obtained in this investigation with those obtained in recent work on soil nitrogen (Bremner, 1949, 1950; Bremner & Shaw, 1954) shows that the similarity in nitrogen distribution and amino acid composition between soil and rotted straw is much greater than between soil and unrotted straw. The proportion of the total nitrogen present as α-amino-nitrogen is generally lower, and the proportion as 'amide-' and amino-sugar-nitrogen higher, in soil than in the well-rotted straw composts examined in this work, but the differences are not very marked. Further work, which will be reported later, has shown that the lignin-like com-

plexes in the oat and wheat straw composts have some of the characteristics of the lignin-like material in soil.

SUMMARY

1. Nitrogen transformations during the decomposition of straw composted with ammonium carbonate have been studied by following the changes in (*a*) the amounts of inorganic and organic nitrogen; (*b*) the amounts of ammonia-, volatile base-, α-amino- and amino sugar-N liberated by acid hydrolysis of the organic nitrogen complexes; and (*c*) the amino acid composition of acid hydrolysates of the composts.

2. Synthesis of organic nitrogen during the biological decomposition of straw composted with ammonium carbonate is not accompanied by any gross change in the distribution of the forms of organic nitrogen. A large fraction of the organic nitrogen synthesized is in the form of protein; a smaller fraction is in the form of amino sugar.

3. The amino acid compositions of acid hydrolysates of rotted and unrotted straws are not greatly different. The rotted, but not the unrotted, straw hydrolysates contain β-alanine and a substance provisionally identified as α, ε-diaminopimelic acid.

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