

## DETERMINATION OF FIXED AMMONIUM IN SOIL

By J. M. BREMNER

*Rothamsted Experimental Station, Harpenden, Herts*

Recent work has shown that many soils have the ability to fix considerable amounts of ammonium and that ammonium-fixing capacity generally increases with depth in the profile. The mechanism of ammonium fixation appears to be the same as that of potassium fixation, the indications being that ammonium replaces interlayer cations ( $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , etc.) in the expanded lattices of clay minerals, and that this causes contraction of the crystal lattices and entrapment of the interlayer ammonium (Barshad, 1948, 1950, 1954*a*, *b*). Several clay minerals including the feldspars (Nash & Marshall, 1957) have been found to fix ammonium, but present evidence indicates that the clay minerals chiefly responsible for ammonium fixation in soil are illite, vermiculite, and montmorillonite. Since many soils contain these clay minerals, it seems likely that they will also contain fixed ammonium due to reaction of the minerals with ammonium either formed naturally or added in the form of fertilizer. Evidence for the presence of fixed ammonium in soil was provided by Rodrigues (1954), who showed that a considerable amount of the nitrogen in some tropical soils was resistant to ordinary base-exchange methods of extraction, hydrogen peroxide oxidation, chromic-sulphuric acid oxidation at 100° C., and boiling 10% sulphuric acid, but was immediately released in the form of ammonium by treatment with hydrofluoric acid. Rodrigues concluded that this ammonium was derived from fixed ammonium in clay minerals and, from estimations of the amounts of ammonium released by hydrofluoric acid treatment of soil profile samples, he deduced that 14–78% of the nitrogen in the samples was in the form of fixed ammonium. He also concluded that the pronounced decrease in C/N ratio with depth in some of the profiles examined could be largely explained by his finding that the proportion of soil nitrogen in the form of fixed ammonium increased markedly with depth in these profiles. Hanway & Scott (1956) used Rodrigues's method and the alkaline distillation technique of Barshad (1951) to determine fixed ammonium in profile samples of Iowa soils, and found that the two methods gave similar results with subsoils but markedly different results with surface soils. Their findings indicated that the soils examined contained significant amounts of fixed ammonium and that the proportion of soil nitrogen in this form increased with depth in the profile.

In Rodrigues's method of determining fixed ammonium, the soil is treated with a 4:1 (v/v) mixture of 40% hydrofluoric acid and 50% sulphuric acid for 1 hr., and the ammonium released by this treatment is estimated by distillation of the extract with alkali after removal of hydrofluoric acid with sulphuric acid. (Rodrigues did not state the strength of the hydrofluoric acid solution he used. It has been assumed that the 40% hydrofluoric acid solution supplied commercially was employed.) This method is open to criticism on the grounds that the techniques used to release fixed ammonium and to estimate the amount released seem likely to convert some of the organically bound soil nitrogen to ammonium-nitrogen. A further difficulty in evaluating this method is that it has not so far been demonstrated that the treatment with hydrofluoric and sulphuric acids effects quantitative release of fixed ammonium from clay minerals.

In the alkaline-distillation method, the soil sample is distilled with NaOH and a duplicate sample is distilled with KOH, and fixed ammonium is estimated from the difference between the amounts of ammonium released by the two distillations. The method is based on Barshad's (1948) finding that fixed ammonium is released from vermiculite by distillation with NaOH but is not released by distillation with KOH. Its value for the determination of fixed ammonium in soil seems limited, since it has been found that distillation of some soils with NaOH does not release all of the fixed ammonium present and that the release of fixed ammonium by distillation with NaOH is blocked effectively by small amounts of potassium (Allison & Roller, 1955; Hanway & Scott, 1956; Hanway, Scott & Stanford, 1957; Nõmmik, 1957). A further criticism of the method is that it involves the dubious assumption that the amount of ammonium released from organic nitrogen compounds on distillation of soil with KOH is the same as that released on distillation with NaOH.

Work previously reported (Bremner & Harada, 1959) indicated that fixed ammonium in soil should be determinable from the difference in the amounts of ammonium released by treatment with N-HF:N-HCl for 24 hr. and by extraction with N-KCl, since the treatment with hydrofluoric acid was found to effect quantitative release of fixed ammonium from clay minerals and evidence was obtained to indicate

that it did not convert significant amounts of organically bound soil nitrogen to ammonium-nitrogen. It was decided therefore to investigate this method further and to compare the results obtained by its application to various soil-profile samples with those obtained by the methods of Rodrigues and Barshad. As shown below, the findings by this method regarding the distribution of fixed ammonium in the soil profiles examined prompted other investigations on the nitrogen compounds in these profiles.

### MATERIALS AND METHODS

*Soils.* The soils used (Table 1) included samples from several profiles known to contain the types of clay minerals believed to be chiefly responsible for ammonium fixation in soil. Samples 1-4 were from the Broadbalk continuous wheat plots at Rothamsted where the soil is derived from clay-with-flints overlying chalk. Sample 1 was from the plot which had received no manure or fertilizer since 1839 (plot 3), sample 2 from the plot receiving complete minerals and nitrogen annually (plot 7), sample 3 from the plot given annual additions of nitrogen, phosphorus, and potassium (plot 13), and sample 4 from the plot receiving farmyard manure annually (plot 2B). Samples 5a, 5b, 5c and 5d were taken in 1893 from a profile of the soil on plot 7 of the Broadbalk field. Sample 6 was from an arable field on the Weald Clay. Samples 7a, 7b, 8a and 8b were from profiles of brown earth soils in Anglesey (7a and 7b, Castleton series; 8a and 8b Arfon series). Sample 9 was from a gley soil profile in Anglesey (Cegin series) and sample 10 from a profile in Rivington, Lancashire (Hallsworth series). Before use the samples were ground to pass a sixty-mesh sieve. The analyses reported in Table 1 were performed by methods previously described (Bremner

& Harada, 1958); information regarding clay mineral composition was provided by Mr G. Brown.

Experiments involving treatment of soil with hydrofluoric acid were carried out with polythene apparatus, and filtrations of mixtures containing this acid were performed with Whatman no. 40 filter-paper. Unless otherwise stated, total-N was determined by a micro-Kjeldahl procedure (Bremner, 1949) and ammonium-N was determined by distillation with MgO at 25° C. (Bremner & Shaw, 1955) or with pH 8.8 sodium borate buffer (Bremner & Shaw, 1954), techniques previously described (Bremner & Harada, 1958) being used where necessary to eliminate interference by hydrofluoric acid.

### RESULTS

#### *Determination of fixed ammonium-N in soils by N-HF:N-HCl method and by method of Rodrigues*

Table 2 shows the amounts of total-, ammonium- and organic-N released by treatment of the soils with N-HF:N-HCl and with the HF-H<sub>2</sub>SO<sub>4</sub> mixture used in Rodrigues's method of determining fixed ammonium in soil. In the treatments with N-HF:N-HCl, 5 g. samples of soil were shaken in polythene bottles with 100 ml. N-HF:N-HCl and after 24 hr. the mixtures were filtered. In the treatments with HF-H<sub>2</sub>SO<sub>4</sub> mixture, 5 g. samples of soil were mixed in polythene bottles with 5 ml. 50% H<sub>2</sub>SO<sub>4</sub> and 20 ml. 40% HF and after 1 hr. the mixtures were treated with 75 ml. water, shaken thoroughly for 5 min. and filtered. Total-N in the extracts was determined by a micro-Kjeldahl procedure previously described (Bremner, 1949); ammonium-N was determined by distillation of neutralized aliquots with pH 8.8 sodium borate buffer (Bremner & Harada, 1958); organic-N was calculated by subtracting ammonium-N from total-N, since the soils contained small amounts of nitrate

Table 1. *Analysis of soils*

Sample	Depth (in.)	Soil	pH	Percentage on moisture-free basis			C/N ratio	Predominant clay minerals*
				C	N	CaCO <sub>3</sub>		
1	0-6	Clay loam	8.2	0.99	0.105	2.1	9.4	M, K
2	0-6	Clay loam	7.9	1.08	0.117	0.6	9.2	M, K
3	0-6	Clay loam	7.7	1.09	0.122	0.6	8.9	M, K
4	0-6	Clay loam	7.8	2.51	0.256	1.4	9.8	M, K
5a	1-9	Clay loam	8.0	1.04	0.122	2.2	8.5	M, K
5b	18-27	—	7.6	0.37	0.055	0.1	6.7	M, K
5c	46-54	—	7.4	0.14	0.031	0.1	4.5	M, K
5d	73-81	—	6.9	0.14	0.031	0.1	4.5	M, K
6	0-6	Silty clay loam	7.5	2.63	0.263	0.2	10.0	M, K
7a	0-6	Sandy loam	5.3	3.10	0.322	0	9.6	V, M, K
7b	20-28	—	5.9	0.14	0.034	0	4.1	M, K
8a	0-9	Sandy loam	6.3	3.68	0.375	0	9.8	V, K
8b	22-26	—	6.4	0.46	0.045	0	10.2	V, M, K
9	13-20	Silty loam	4.8	2.15	0.175	0	12.3	V, M, K
10	14-24	Clay	4.6	1.78	0.198	0	9.0	V

\* M, mica type (e.g. illite); K, kaolin type; V, vermiculite type.

and only a little, if any, of this would be recovered by the method used to determine total-N. Experiments with the HF-HCl extracts of soils 1-4 showed that the results obtained by the borate buffer method of determining ammonium-N were in close agreement with those obtained by distillation with MgO at 25° C., and that ammonium-N added to these extracts was recovered quantitatively by both methods.

Table 3 gives the amounts of exchangeable ammonium-N in the soils as determined by extraction with N-KCl and with N-KCl at pH 1, and the amounts of fixed ammonium-N as estimated by subtracting ammonium-N extracted by N-KCl from ammonium-N released by the treatments with hydrofluoric acid. Exchangeable ammonium-N was determined by shaking 10 g. samples of soil for 1 hr. with 100 ml. N-KCl or with 100 ml. N-KCl containing sufficient HCl to give the suspension a pH value of about 1.0 (Olsen, 1929) and determining ammonium-N in the extracts by distillation with borate buffer.

*Determination of ammonium-N in HF-HCl and HF-H<sub>2</sub>SO<sub>4</sub> extracts of soils*

In the procedure used by Rodrigues for the determination of fixed ammonium, the soil was treated with HF and H<sub>2</sub>SO<sub>4</sub> as described above and after standing 1 hr. the supernatant liquid was decanted and the residue washed with water in a centrifuge. Ammonium-N in the extract was then estimated by distilling an aliquot with alkali (presumably NaOH) in the Markham (1942) distillation apparatus 'after driving off the hydrofluoric acid in the presence of sulphuric acid'. The treatment with sulphuric acid was presumably designed to eliminate interference by HF in the determination of ammonium-N (see

Bremner & Harada, 1958). This procedure was not followed in the work reported in Table 2, since it seemed very likely that treatment of the soil extracts with sulphuric acid followed by distillation with NaOH would lead to the formation of considerable amounts of ammonium-N by decomposition of organic nitrogen compounds. To examine this possibility and to compare different methods of estimating ammonium-N, the soils were treated with HF-HCl mixture and with HF-H<sub>2</sub>SO<sub>4</sub> mixture as described in the previous section, and the ammonium-N contents of the extracts were estimated by the following methods: A, distillation with NaOH after removal of HF with H<sub>2</sub>SO<sub>4</sub>; B, distillation with NaOH; C, distillation with MgO; D, distillation with sodium borate buffer (pH 8.8); E, distillation with MgO at 25° C. In method A, 5 ml. aliquots of the extracts were heated with 1 ml. conc. H<sub>2</sub>SO<sub>4</sub> in micro-Kjeldahl digestion flasks to drive off the HF and then distilled with 7 ml. 10N-NaOH in the Markham apparatus. In methods B, C and D, 25-50 ml. aliquots of the extracts were pipetted into 500 ml. Kjeldahl flasks, brought to pH 6.3 by cautious addition of 2N-NaOH using methyl red as indicator, made alkaline with the appropriate reagent (B, 10 ml. 10N-NaOH; C, 5 g. carbonate-free light MgO; D, 50 ml. of a solution containing 8 g. boric acid and 40 g. sodium borate per litre) and treated with sufficient water to give a total volume of 300 ml. The flasks were then stoppered and shaken for a few minutes before connexion to the distillation apparatus (macro-Kjeldahl assembly). The NH<sub>3</sub> liberated by boiling (30 min.) was collected in boric acid and titrated with 0.01N-H<sub>2</sub>SO<sub>4</sub>. The extracts were neutralized with NaOH before addition of the alkaline reagent and shaken after addition of this reagent in order to eliminate interference by HF in

Table 2. Total-N, ammonium-N and organic-N in HF-HCl and HF-H<sub>2</sub>SO<sub>4</sub> extracts of soils

Soil	N in extract (% total soil N)					
	HF-HCl extract			HF-H <sub>2</sub> SO <sub>4</sub> extract		
	Total	Ammonium*	Organic	Total	Ammonium*	Organic
1	23.3	6.3 (27.0)	17.0	40.4	8.6 (21.2)	31.8
2	28.6	8.1 (28.3)	20.5	39.0	10.4 (26.6)	28.6
3	26.5	7.6 (28.6)	18.9	40.2	10.9 (27.1)	29.3
4	15.5	4.3 (27.7)	11.2	25.2	5.1 (20.2)	20.1
5a	26.4	7.1 (26.9)	19.3	40.3	9.5 (23.5)	30.8
5b	41.0	19.3 (47.0)	21.7	60.1	28.0 (46.7)	32.1
5c	66.1	23.0 (34.8)	43.1	66.9	23.2 (34.6)	43.7
5d	67.9	23.0 (33.8)	44.9	70.0	23.9 (34.1)	46.1
6	19.3	5.9 (30.5)	13.4	34.1	8.0 (23.5)	26.1
7a	25.4	6.4 (25.2)	19.0	38.1	6.6 (17.3)	31.5
7b	55.8	46.0 (82.2)	9.8	53.1	27.4 (51.6)	25.7
8a	20.6	3.3 (16.0)	17.3	38.0	4.1 (10.8)	33.9
8b	43.2	18.7 (43.3)	24.5	75.0	30.2 (40.2)	44.8
9	27.6	18.4 (66.7)	9.2	67.7	25.6 (37.8)	42.1
10	30.0	10.5 (35.0)	19.5	48.7	12.3 (25.2)	36.4

\* Figures in parentheses represent ammonium-N as percentage of total-N in extract.

Table 3. Exchangeable and fixed ammonium nitrogen in soils as estimated by different methods  
Exchangeable ammonium-N estimated by extraction with N-KCl and with N-KCl acidified with HCl (pH 1.0).

Soil	Fixed ammonium-N														
	NH <sub>4</sub> -N released (p.p.m.)					HF-HCl method					HF-H <sub>2</sub> SO <sub>4</sub> method				
	Total-N content (p.p.m.)	HF-HCl (a)	HF-H <sub>2</sub> SO <sub>4</sub> (b)	N-KCl (c)	N-KCl:HCl	P.p.m.* (a-c)	M-equiv.†	% soil N	P.p.m.* (b-c)	M-equiv.†	% soil N	Total soil N	P.p.m.* (a-c)	M-equiv.†	% soil N
1	1050	66	90	3	3	63	0.45	6.0	87	0.62	8.3	87	0.62	8.3	87
2	1170	95	122	6	6	89	0.64	7.6	116	0.83	9.9	116	0.83	9.9	116
3	1220	93	133	2	3	91	0.65	7.5	131	0.94	10.8	131	0.94	10.8	131
4	2560	110	130	11	11	99	0.71	3.9	119	0.85	4.7	119	0.85	4.7	119
5a	1220	87	116	19	19	68	0.49	5.6	97	0.69	8.0	97	0.69	8.0	97
5b	550	106	154	5	5	101	0.72	18.4	149	1.06	27.1	149	1.06	27.1	149
5c	314	72	73	1	1	71	0.51	22.6	72	0.51	22.9	72	0.51	22.9	72
5d	313	72	75	1	1	71	0.51	22.7	74	0.53	23.6	74	0.53	23.6	74
6	2630	155	210	11	12	144	1.03	5.5	199	1.42	7.6	199	1.42	7.6	199
7a	3220	206	212	23	24	183	1.31	5.7	189	1.35	5.9	189	1.35	5.9	189
7b	344	158	94	8	9	150	1.07	43.7	86	0.61	25.0	86	0.61	25.0	86
8a	3750	124	154	20	21	104	0.74	2.8	134	0.96	3.6	134	0.96	3.6	134
8b	447	84	135	10	11	74	0.53	16.5	125	0.89	28.0	125	0.89	28.0	125
9	1750	322	447	19	20	303	2.16	17.3	428	3.06	24.5	428	3.06	24.5	428
10	1980	208	244	26	27	182	1.30	9.2	218	1.56	11.0	218	1.56	11.0	218

\* 1 p.p.m. is approximately equivalent to 2 lb./acre. † Milliequivalents/100 g. of soil.

the determination of ammonium-N (see Bremner & Harada, 1959). In method E, interference by HF was eliminated by diluting the aliquots of HF-HCl extracts taken for analysis with an equal volume of water. Ammonium-N in ammonium sulphate solutions made 1 N with respect to both HF and HCl was found to be recovered quantitatively by methods B-E (method A was not tested). It can be seen that method A gave much higher values than the other methods tested and that methods D and E gave very similar results.

Determination of fixed ammonium-N in soils by alkaline distillation method

Table 5 gives the results obtained when fixed ammonium-N in some of the soils was determined by the alkaline distillation method. The rapid procedure described by Allison & Roller (1955) was used, 5 g. samples of soil being distilled with 500 ml. 0.2N-alkali until 300 ml. of distillate had been collected. Serious bumping and frothing occurred during distillation of some of the soils, but it was found that this could be eliminated by addition of glass beads and mineral oil and by careful regulation of the rate of distillation.

Since several workers (Allison & Roller, 1955; Hanway & Scott, 1956; Hanway *et al.* 1957; Nömmik, 1957) have shown that the release of fixed ammonium by distillation with NaOH is blocked by small amounts of potassium, the effect of leaching soils with N-NaCl to remove exchangeable potassium before distillation with alkali was studied. In this investigation, 5 g. samples of soils 5b, 5c, 5d and 6 were leached with 200 ml. N-NaCl and the leached samples were immediately transferred to 800 ml. Kjeldahl flasks and distilled with alkali. The small amounts of fixed ammonium-N removed from the soils by leaching with N-NaCl were determined (NH<sub>4</sub>-N in N-NaCl extract minus NH<sub>4</sub>-N in N-KCl extract) and added to the amounts estimated by distillation of the leached soils with NaOH and KOH. The results obtained by this procedure indicated that the amounts of fixed ammonium-N in the soils, calculated as percentages of total soil N, were as follows: 5b, 2.1%; 5c, 3.2%; 5d, 3.1%; 6, 1.1%. The NaCl-treated samples were not allowed to dry before distillation, since several workers (e.g. Attoe, 1947; Scott, Hanway & Stickney, 1957) have shown that air drying of soil can lead to a marked increase in its content of exchangeable potassium.

Effect of KOH pretreatment on release of ammonium-N from soil by N-HF:N-HCl

Although work previously reported (Bremner & Harada, 1958) indicated that the results obtained by the N-HF:N-HCl method of determining fixed ammonium in soil were not likely to be seriously

Table 4. Ammonium-N in HF-HCl and HF-H<sub>2</sub>SO<sub>4</sub> extracts of soils as estimated by various methods

Ammonium-N in extracts estimated by distillation with: A, NaOH (after removal of HF with H<sub>2</sub>SO<sub>4</sub>); B, NaOH; C, MgO; D, borate buffer (pH 8.8); E, MgO at 25° C.

Method	...	Ammonium-N (% total soil N)								
		HF-HCl extract					HF-H <sub>2</sub> SO <sub>4</sub> extract			
		A	B	C	D	E	A	B	D	
Soil 1		20.6	9.6	6.6	6.2	6.1	36.4	13.3	8.8	
2		—	10.6	8.4	8.2	8.0	35.6	13.7	10.2	
3		—	10.4	8.0	7.8	7.6	37.8	13.8	10.6	
4		—	5.4	4.7	4.3	4.2	21.8	8.3	5.0	
5a		—	10.4	—	7.4	7.2	36.8	13.3	9.8	
5b		35.8	22.2	—	19.5	19.1	—	31.2	27.4	
5c		—	29.8	—	23.0	22.8	—	29.2	23.9	
5d		50.6	29.0	—	23.4	23.0	58.8	30.7	24.1	
6		17.9	8.8	6.1	5.7	5.7	29.6	12.5	7.7	
7a		—	9.6	6.6	6.3	6.3	—	10.6	6.9	
7b		54.1	50.6	46.5	46.1	45.8	51.2	32.1	28.5	
8a		—	5.5	—	3.3	3.2	—	8.1	4.4	
8b		38.6	23.9	—	18.3	18.1	64.9	33.7	29.6	
9		—	24.6	—	18.2	18.0	—	30.3	25.0	
10		26.2	14.3	10.7	10.6	10.4	38.2	17.6	12.9	

Table 5. Fixed ammonium nitrogen in soils as estimated by alkaline distillation method

Soil	NH <sub>3</sub> -N liberated*		
	NaOH distillation	KOH distillation	Fixed ammonium-N*
	(a)	(b)	(a-b)
2	12.3	11.5	0.8
4	11.4	10.8	0.6
5b	11.5	10.0	1.5
5c	11.5	8.3	3.2
5d	11.7	8.6	3.1
6	12.1	11.4	0.7
7b	10.6	8.8	1.8

\* % total soil N.

Table 6. Effect of KOH pretreatment on release of ammonium-N from soils by N-HF:N-HCl

Soil	NH <sub>4</sub> -N released by N-HF:N-HCl (% total soil N)	
	Before pretreatment	After pretreatment
	5d	21.9
6	5.4	5.0
7a	5.5	6.1
8b	16.0	17.1
10	9.0	8.2

affected by decomposition of organically bound soil nitrogen to ammonium-N during the acid treatment used to release fixed ammonium, it was decided to investigate this question further by studying the effect of pretreating soils with hot KOH solution on the release of ammonium-N by N-HF:N-HCl. This pretreatment should not remove fixed ammonium from clay minerals but should greatly reduce interference in the N-HF:N-HCl method by nitrogenous

organic materials, since hot KOH dissolves a considerable fraction of soil organic matter and decomposes labile nitrogen compounds such as glutamine, asparagine and glucosamine. A marked decrease in the amount of ammonium-N released by N-HF:N-HCl treatment of soil as a result of pretreatment with KOH would therefore indicate that significant decomposition of organically bound nitrogen to ammonium-N occurred on direct treatment with N-HF:N-HCl.

The procedure adopted was as follows. 5 g. samples of soil were heated with 50 ml. 0.5 N-KOH in 100 ml. beakers until about half of the liquid had evaporated. The contents of the beakers were then transferred to polythene centrifuge tubes and the residues from the KOH treatment were separated by centrifugation, washed three times on the centrifuge with KCl solution (twice with 100 ml. N-KCl and once with 100 ml. 0.5 N-KCl) and dried *in vacuo* over KOH at room temperature. The residues were then shaken with N-HF:N-HCl (100 ml.) for 24 hr., the mixtures were filtered, and ammonium-N in the extracts was determined by distillation with MgO at 25° C. Control experiments in which the soils were not treated with KOH, but were extracted with KCl before HF-HCl treatment were performed simultaneously. The results are given in Table 6. It can be seen that pretreatment of the soils with hot KOH had little effect on the release of ammonium-N by N-HF:N-HCl.

*Release of ammonium-N on treatment of soils with N-HF:N-HCl. Effects of varying period of treatment and volume of reagent*

Table 7 shows the amounts of ammonium-N released by shaking 5 g. samples of soil with 100 ml.

N-HF:N-HCl for various periods of time, and Table 8 shows the amounts released by shaking 5 g. samples with different volumes of N-HF:N-HCl for 24 hr. Ammonium-N was determined by distillation with pH 8.8 borate buffer.

*Release of nitrogen by acid hydrolysis of soils and by treatment of hydrolysis residues with hydrofluoric acid*

Table 9 gives the amounts of total-, ammonium- and  $\alpha$ -amino-N liberated by hydrolysis of the soils with 6N-HCl and the amounts of ammonium-N released by treatment of the hydrolysis residues with N-HF:N-HCl. The soils were hydrolysed by boiling with 6N-HCl (3 ml./g. of soil) under reflux for 12 hr., a few drops of capryl alcohol being added to prevent frothing. The hydrolysis mixtures were filtered through weighed sinter-glass funnels with suction, the residues were washed thoroughly with water, and the filtrates were evaporated *in vacuo* to remove HCl and made to volume. The residues were dried to constant weight *in vacuo* over KOH at room temperature and samples of the dried materials were taken for treatment with N-HF:N-HCl (20 ml./g. of residue) at room temperature for 24 hr. Ammonium-N released by hydrolysis and by treatment of the hydrolysis residues with N-HF:N-HCl was determined by distillation with MgO at 25° C.;  $\alpha$ -amino-N in the hydrolysates was determined by the manometric ninhydrin method of Van Slyke, Dillon, MacFadyen & Hamilton (1941) as previously described (Bremner, 1949). From 14 to 20% of the

material in the soils was dissolved by the hydrolysis with 6N-HCl.

*Extraction of nitrogen from soils by neutral pyrophosphate and alkali*

Table 10 shows the amounts of total- and ammonium-N released by shaking 10 g. samples of soil profile samples with 100 ml. 0.5N-sodium hydroxide and with 100 ml. 0.1M-sodium potassium pyrophosphate (pH 7.0) at room temperature for 24 hr. The neutral pyrophosphate solution was prepared by mixing 0.1M- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$  with 0.1M- $\text{K}_4\text{P}_2\text{O}_7$ . Ammonium-N in the extracts was determined by distillation with MgO at 25° C.

*Determination of total nitrogen in soils by Kjeldahl method*

In the analyses reported in Table 1, total-N in the soils was determined by the Kjeldahl method using the pretreatment with water recommended by Bal (1925) for the determination of nitrogen in heavy clay soils. The results obtained in a study of the effect of this pretreatment are given in Table 11. In the method in which the pretreatment with water was omitted, the soil (10 g.) was treated with 10 g.  $\text{K}_2\text{SO}_4$ , 1 g.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.1 g. Se and 40 ml. conc.  $\text{H}_2\text{SO}_4$ , and the mixture was boiled gently for 5 hr. In the method which included the pretreatment, the soil (10 g.) was treated with water (25 ml.) and after shaking for 30 min. the mixture was treated with 40 ml. conc.  $\text{H}_2\text{SO}_4$  and heated until the water was removed. The mixture was then cooled, treated

Table 7. Release of ammonium-N on treatment of soils with N-HF:N-HCl. Effect of varying period of treatment

Period of treatment (hr.) ...	Ammonium-N released (% total soil N)*					
	2	6	12	24	48	96
Soil 2	6.1	7.0 (26.0)	8.0	8.2 (28.9)	8.4	8.7 (33.0)
4	3.0	3.4 (13.3)	3.9	4.3 (15.7)	4.6	4.9 (19.9)
5a	5.3	6.2 (22.9)	6.8	7.2 (26.8)	8.0	8.4 (30.2)
5b	10.2	15.1 (33.2)	18.0	19.6 (41.6)	20.6	22.0 (48.5)
7b	17.0	26.9 (36.0)	37.8	45.6 (55.6)	57.0	64.2 (77.0)
9	10.2	14.0 (22.2)	16.9	18.6 (28.0)	19.0	19.3 (30.0)

\* Figures in parentheses represent percentages of total soil N released by treatment.

Table 8. Release of ammonium-N on treatment of soils with N-HF:N-HCl. Effect of varying volume of reagent

Ml. reagent per g. of soil ...	Ammonium-N released (% total soil N)				
	10	20	30	40	50
Soil 2	5.3	8.2	8.2	8.1	8.3
4	3.0	4.2	4.1	4.3	4.5
5b	13.1	19.9	20.6	22.3	23.9
6	3.6	5.9	6.0	5.9	6.1
7a	4.3	6.4	6.5	6.4	6.7
7b	34.5	48.0	52.1	53.5	58.7
8a	2.3	3.3	3.5	3.4	3.6
9	10.0	18.9	20.9	21.6	22.3

Table 9. Amounts of nitrogen released by hydrolysis of soils with 6N-HCl and by treatment of hydrolysis residues with N-HF:N-HCl

(Results expressed as percentages of total nitrogen in soils.)

Soil	N released by 6N-HCl hydrolysis of soil			NH <sub>4</sub> -N released by treatment with N-HF:N-HCl	
	Total	$\alpha$ -Amino	Ammonium*	Soil	Hydrolysis residue
1	78.2	—	21.0 (26.8)	6.3	1.7
2	75.6	—	20.8 (27.5)	8.1	2.7
3	77.5	—	21.5 (27.7)	7.6	1.8
4	75.4	—	19.6 (26.0)	4.3	1.7
5a	80.2	32.9	22.2 (27.7)	7.1	2.3
5b	58.8	—	25.6 (43.5)	19.3	1.9
5c	46.0	12.1	24.9 (54.1)	23.0	3.6
5d	40.8	—	23.7 (58.1)	23.0	3.6
6	81.9	34.6	20.8 (25.4)	5.9	2.1
7a	76.3	33.1	22.0 (28.8)	6.4	1.3
7b	47.0	5.1	36.6 (77.9)	46.0	24.1
8a	74.0	32.2	18.5 (25.0)	3.3	1.0
8b	65.2	19.8	26.5 (40.6)	18.7	5.4
9	59.6	—	21.2 (35.6)	18.4	8.2
10	66.9	—	22.3 (33.3)	10.5	2.9

\* Figures in parentheses represent ammonium-N released by hydrolysis as percentage of total-N released by hydrolysis.

Table 10. Total-N, ammonium-N and organic-N in neutral pyrophosphate and alkali extracts of soils

Soil	N in extract (% total soil N)					
	0.5N-sodium hydroxide			0.1M-NaK pyrophosphate		
	Total	Ammonium	Organic	Total	Ammonium	Organic
5a	33.0	4.0	29.0	15.5	1.8	13.7
5b	24.0	1.0	23.0	9.4	1.0	8.4
5c	14.4	1.3	13.1	4.9	1.0	3.9
5d	14.4	1.3	13.1	3.9	0.8	3.1
7a	60.5	2.4	58.1	23.6	1.1	22.5
7b	15.7	2.8	12.9	12.9	2.7	10.2
8a	52.5	4.9	47.6	20.9	1.6	19.3
8b	44.1	5.0	39.1	19.9	3.5	16.4

with 10 g. K<sub>2</sub>SO<sub>4</sub>, 1 g. CuSO<sub>4</sub>·5H<sub>2</sub>O and 0.1 g. Se and boiled gently for 5 hr. Ammonium-N in the digests was determined by distillation with NaOH.

To facilitate comparison of the results obtained with the surface soils and subsoils used in this work, the analytical data concerning these samples have been summarized in Table 12.

DISCUSSION

Comparison of the results given in Tables 2 and 12 shows that, with one exception (soil 7b), treatment of the soils with the HF-sulphuric acid mixture used in Rodrigues's method of determining fixed ammonium in soil released more ammonium and considerably more organic nitrogen than did treatment with N-HF:N-HCl. Since the latter treatment effects quantitative release of fixed ammonium from clay minerals (Bremner & Harada, 1958), this finding indicates that treatment of soil with the

strongly acidic HF-sulphuric acid mixture (approx. 18.4N with respect to HF and 3.6N with respect to H<sub>2</sub>SO<sub>4</sub>) causes some hydrolysis and decomposition of soil organic nitrogen compounds with formation of acid-soluble organic-N and ammonium-N. The possibility that such hydrolysis and decomposition occurs is increased by the fact that considerable heat is evolved on treatment of soil with Rodrigues's mixture. For example, it was found that the heat generated when 1 g. samples of soils 1, 3, 6 and 10 were treated with 5 ml. of this mixture raised the temperatures of the soil-acid suspensions to 55–60° C. The temperature developed apparently depends upon the amount and type of clay mineral present: temperatures of 70–95° C. were recorded when 1 g. samples of illite, vermiculite, and bentonite were treated with 5 ml. of Rodrigues's mixture. No development of heat could be detected on treatment of mineral soils with N-HF:N-HCl.

The results in Tables 3 and 12 show that the proportion of soil nitrogen in the form of fixed ammonium, as determined by the N-HF:N-HCl method, was much higher in the subsoils than in the surface soils examined (cf. Rodrigues, 1954), but that the total amount of fixed ammonium actually decreased with depth in some of the profiles examined although it was higher on the average in the subsoils than in the surface soils. Calculated as percentages of total soil nitrogen, the amounts of organic-N released by treatment of the profile samples with hydrofluoric acid increased markedly with depth in the profile (Tables 2 and 12). The soil with the highest fixed ammonium content (sample 9) also had the highest clay content (42 %).

The results in Table 4 show that the method used by Rodrigues to estimate ammonium-N in HF-sulphuric acid extracts of soil causes extensive decomposition of organic nitrogen compounds to ammonium-N and gives values often three to four times greater than those obtained by the borate buffer method of determining ammonium-N. The finding that the borate buffer method gave practically the same results as the mild MgO micro-diffusion method with N-HF:N-HCl extracts of soil, indicates that it does not cause significant decomposition of organic nitrogen compounds in these extracts to ammonium. It also shows that treatment of soil with N-HF:N-HCl does not release significant amounts of free amino sugars by hydrolysis of amino sugar complexes, since previous work (Bremner & Shaw, 1954) established that glucosamine is decomposed practically quantitatively to ammonia when distilled with pH 8.8 borate buffer, but is not significantly decomposed to ammonia when distilled with MgO at 25° C. Comparison of the results in Tables 3 and 4 shows that the values obtained by Rodrigues's method of determining fixed ammonium when it was modified by using the borate buffer method to estimate ammonium released by treatment with hydrofluoric and sulphuric acid, were often very similar to those obtained by the N-HF:N-HCl method.

No agreement exists at present regarding the most satisfactory method of determining 'exchangeable' ammonium in soil (see Harmsen & Van Schreven, 1955). In light of the information now available

Table 11. Effect of pretreatment with water on determination of nitrogen in soils by the Kjeldahl method

Soil	N content (%)	
	Untreated soil	Treated soil
1	0.105	0.105
2	0.116	0.117
4	0.256	0.256
5a	0.121	0.122
5b	0.055	0.055
6	0.262	0.263
7a	0.320	0.322
7b	0.034	0.034
8a	0.373	0.375
8b	0.045	0.045
9	0.174	0.175

Table 12. Summary of analytical data on surface soils and subsoils

		Surface soils		Subsoils	
		Nitrogen released (% total soil N)			
		Average	Range	Average	Range
N-HF:N-HCl	Total	23.2	15.5-28.6	47.4	27.6-67.9
	Ammonium	6.1	3.3-8.1	22.7	10.5-46.0
	Organic	17.1	11.2-20.5	24.7	9.2-44.9
HF-H <sub>2</sub> SO <sub>4</sub>	Total	36.9	25.2-40.4	63.1	48.7-75.0
	Ammonium	7.9	4.1-10.9	24.4	12.3-30.2
	Organic	29.0	20.1-33.9	38.7	25.7-46.1
6N-HCl hydrolysis	Total	77.4	74.0-81.9	54.9	40.8-66.9
	Ammonium	20.8	18.5-22.2	25.8	21.2-36.6
	Organic	56.6	54.3-61.1	29.1	10.4-44.6
0.5N-NaOH	Total	48.7	33.0-60.5	22.5	14.4-44.1
	Ammonium	3.8	2.4-4.9	2.3	1.0-5.0
	Organic	44.9	29.0-58.1	20.2	12.9-39.1
0.1M-NaK pyrophosphate	Total	20.0	15.5-23.6	10.2	3.9-19.9
	Ammonium	1.5	1.1-1.8	1.8	0.8-3.5
	Organic	18.5	13.7-22.5	8.4	3.1-16.4
N-KCl	Ammonium	0.5	0.2-1.6	1.2	0.3-2.3
Fixed ammonium-N					
HF-HCl method	% total soil N	5.6	2.8-7.6	21.5	9.2-43.7
	M-equiv./100 g. soil	0.70	0.45-1.31	0.97	0.51-2.16
HF-H <sub>2</sub> SO <sub>4</sub> method	% total soil N	7.4	3.6-10.8	23.2	11.0-28.0
	M-equiv./100 g. soil	0.97	0.62-1.42	1.20	0.51-3.06



regarding fixed ammonium in soil, it is clear that in considering the subject of exchangeable ammonium the matter of definition is of considerable importance. Since fixed ammonium is apparently best defined as ammonium that is not extractable by *N*-KCl (Allison & Roller, 1955), it appears logical that exchangeable ammonium be henceforth defined as ammonium that is extractable by *N*-KCl. Many workers have found that in practice it is more convenient to determine exchangeable ammonium by the method of Olsen (1929) in which the soil is extracted with *N*-KCl containing sufficient HCl to give the suspension a pH value of about 1.0. It is important therefore to know if the results obtained by this method are comparable to those obtained by extraction with *N*-KCl. Harmsen & Van Schreven (1955) state that the results obtained by Olsen's method are not comparable to those obtained by extraction at a neutral reaction, but do not indicate the basis for this statement. Similarly, Chaminade (1940) has provided no basis for his statement that more ammonium is extracted from soil by Olsen's method than by the neutral potassium chloride technique because the acid solution attacks the mineral colloids and releases some of the non-exchangeable ammonium. In previous work in this laboratory it was found that the two methods gave very similar results when applied to surface soils, but it was not known if the soils examined contained significant amounts of fixed ammonium. However, the results in Table 3 show that the two methods gave very similar results even with soils containing large amounts of fixed ammonium. Subsequent work has shown that more ammonium is extracted from clay minerals containing fixed ammonium by *N*-KCl at pH 1.0 than by neutral *N*-KCl, but until it is demonstrated that this holds for soils as well as clay minerals the use of Olsen's method of determining exchangeable ammonium in soil would appear justifiable.

Comparison of the results in Tables 3 and 5 shows that the values obtained when fixed ammonium in the soils was determined by the alkaline distillation method were much lower than those obtained by the hydrofluoric acid methods. The finding that leaching of the soils with sodium chloride solution to remove exchangeable potassium before distillation with alkali had little effect on the results obtained by the alkaline distillation method does not exclude the possibility that the low values given by this method were due to interference by potassium, since it seems highly probable that fixed potassium is released on distillation of soil with sodium hydroxide. Nömmik (1957) recently modified the alkaline distillation method by adding a large amount of sodium chloride to reduce interference by potassium during distillation of soil with sodium hydroxide, but the value of this modification has not been established. Apart

from the fact that it is subject to interference by potassium, the alkaline distillation method has the defect that it involves the assumption that the amount of ammonium released from nitrogenous organic material on distillation of soil with KOH solution is practically the same as the amount released on distillation with NaOH solution of equivalent concentration. The validity of this assumption is questionable, since any organic nitrogen compounds within the crystal lattices of clay minerals in soil should be more susceptible to decomposition by NaOH than by KOH. Even if mineral soils do not contain such lattice-bound organic nitrogen compounds, the assumption that the same amount of ammonium is liberated by distillation of soil with NaOH as by distillation with KOH is clearly not justifiable unless care is taken to ensure that the rates of distillation with the two alkalis are practically identical. Considerable trouble was taken to meet this requirement in the analyses reported in Table 5 and fairly consistent results were obtained. In some analyses in which this requirement was not met rigorously it was found that more ammonium was released by distillation with KOH than by distillation with NaOH. Another criticism of the alkaline distillation method is that there does not appear to be any evidence that ammonium fixed by illite is recovered quantitatively by distillation with NaOH.

The finding that pretreatment of soil with hot potassium hydroxide solution to remove organic matter and decompose labile organic nitrogen compounds did not significantly affect the results obtained by the *N*-HF:*N*-HCl method (Table 6) supports previous evidence (Bremner & Harada, 1958) that treatment of soil with *N*-HF:*N*-HCl does not cause serious decomposition of organically bound soil nitrogen to ammonium-N. However, it does not establish conclusively that such decomposition does not occur, since it is possible that the soils examined contained very acid-labile organic nitrogen compounds within the lattices of clay minerals, and that these compounds were not removed by the treatment with KOH, but were released and extensively decomposed to ammonium on treatment with hydrofluoric acid. This possibility cannot be excluded, but it seems very unlikely that soils contain significant amounts of such extremely acid-labile organic nitrogen compounds. Experiments previously reported (Bremner & Harada, 1958) showed that less than 1% of the nitrogen in organic soils was converted to ammonium-N by treatment with *N*-HF:*N*-HCl at room temperature for 24 hr. The possibility that some of the ammonium liberated from soil organic matter during pretreatment of the soils with KOH was fixed by clay minerals seems very remote, since even if the soils had the capacity to fix ammonium under moist conditions,

ammonium fixation should not occur in the presence of such a large amount of fixable potassium. Experiments showed that of the soils used in this work only samples 5*b*, 5*c* and 5*d* fixed ammonium under moist conditions.

Since work previously reported (Bremner & Harada, 1958) showed that the release of fixed ammonium on treatment of clay minerals with *N*-HF:*N*-HCl was complete in 24 hr., the finding (Table 7) that the amount of ammonium released by *N*-HF:*N*-HCl treatment of one of the soils (7*b*) increased very markedly with period of treatment beyond 24 hr., suggested that *N*-HF:*N*-HCl treatment of this soil caused serious decomposition of organic nitrogen compounds to ammonium. However, the subsequent findings with this soil that the amount of organic nitrogen released by *N*-HF:*N*-HCl treatment also increased with period of treatment beyond 24 hr. (Table 7), that the amount of ammonium-N released in 24 hr. increased markedly with the volume of *N*-HF:*N*-HCl employed (Table 8), and that the amount of ammonium-N released by hydrolysis with 6*N*-HCl was considerably less than the amount released by treatment with *N*-HF:*N*-HCl (Table 9), all suggest that the anomalous results obtained with this soil were not due to decomposition of organic nitrogen compounds, but to the fact that treatment with *N*-HF:*N*-HCl for 24 hr. using 20 ml. reagent per gramme of soil did not effect complete release of fixed ammonium and clay-bound organic nitrogen compounds. The results in Tables 7 and 8 also suggest that this treatment was not completely effective for the release of fixed ammonium from the other subsoils (5*b* and 9) used in these experiments.

Mattson & Koutler-Andersson (1943) have shown that lignin can fix an appreciable quantity of ammonia in a chemically stable form, and they have advanced the theory that soil humic acids are derived from plant lignins by autoxidation and ammonia fixation. They have also suggested that the decrease in C/N ratio with depth in soil profiles is due to the presence of relatively greater amounts of nitrogen-rich humic acids in subsoils than in surface soils (Mattson & Koutler-Andersson, 1941). The possibility that some of the ammonium released by hydrofluoric acid treatment of mineral soils may be derived from lignin-ammonia complexes was investigated using lignin-ammonia complexes studied in previous work (Bremner & Shaw, 1957). These complexes had been prepared from commercial wood lignins (Meadol MRM, 0.12% N; Indulin A, 0.08% N) by a method similar to that described by Mattson & Koutler-Andersson (1943) and subsequently washed thoroughly with 0.5*N*-hydrochloric acid to remove adsorbed and exchangeable ammonium. The dried products contained 2.4–3.0% nitrogen, about half of which was resistant to pro-

longed treatment with boiling 6*N*-HCl or 2*N*-NaOH. It was found that less than 1% of the total nitrogen in these complexes was released as ammonium by treatment with *N*-HF:*N*-HCl at room temperature for 24 hr. or with Rodrigues's HF-sulphuric acid mixture for 1 hr. It may be concluded therefore that hydrofluoric acid has no special ability to release ammonia fixed by lignin. The explanation advanced by Mattson & Koutler-Andersson to account for the low C/N ratios of subsoils was examined by analysing 0.1*M*-sodium potassium pyrophosphate (pH 7.0) and 0.5*N*-sodium hydroxide extracts of sample 7*a* (C/N ratio, 9.6) and sample 7*b* (C/N ratio, 4.1). It was found that the humic-N/fulvic-N ratios of the subsoil (7*b*) extracts were much lower than those of the corresponding surface soil (7*a*) extracts, and that the C/N ratios of the humic fractions of the surface soil extracts were slightly lower than those of the humic fractions of the corresponding subsoil extracts. Analysis of the extracts obtained in the experiments reported in Table 10 also showed that the C/N ratios of unfractionated pyrophosphate and alkali extracts of soil profile samples tended to increase with depth in the profile. In these experiments carbon was determined by the method of Tinsley (1950). Taken with the finding that the proportion of soil nitrogen dissolved by pyrophosphate or alkali decreased markedly with depth in the profile (Table 10), these results show that the explanation advanced by Mattson & Koutler-Andersson to account for the decrease in C/N ratio with depth in soil profiles is not acceptable. They also confirm that the ammonium released on treatment of mineral soils with hydrofluoric acid is not derived to any significant extent from lignin-ammonia complexes (or other nitrogenous organic material) in the humic fraction of soil organic matter. If this were not true, the proportion of total soil nitrogen released as ammonium by hydrofluoric acid treatment of sample 7*a*, which has a high percentage of humic-N, would be much higher than the corresponding proportion with sample 7*b*, which has a very low percentage of humic-N, whereas it was actually found to be much lower (see Table 2). Further evidence that very little of the ammonium released on treatment of soil with hydrofluoric acid is derived from the humic fraction of soil organic matter has been previously reported (Bremner & Harada, 1959).

It has been repeatedly observed in studies on the chemical nature of soil nitrogen that a considerable fraction—as much as 30%—of the nitrogen in mineral soils is released as ammonium by acid hydrolysis. The origin of this ammonium has been the subject of much speculation, since the amount liberated is far in excess of the quantity that can be satisfactorily accounted for by deamination of nitrogenous organic materials known to occur in soil (see Bremner, 1949; Bremner & Shaw, 1954;

Stevenson, 1954, 1956). The findings in this work regarding the distribution of fixed ammonium in soil profiles suggested that some of the ammonium liberated by hydrolysis of mineral soils is derived from fixed ammonium in clay minerals, and that the proportion of soil nitrogen liberated as ammonium by hydrolysis should increase with depth in the profile. These deductions are confirmed by the results in Tables 9 and 12, which show that a considerable amount of the fixed ammonium in the soils examined was released by hydrolysis with 6N-HCl and that the proportion of soil nitrogen liberated as ammonium by acid hydrolysis was greater with subsoils than with surface soils. The results in Tables 9 and 12 also show that, calculated as percentages of total soil nitrogen, the amounts of total- and  $\alpha$ -amino-N (amino-acid-N) released by acid hydrolysis of soil profile samples decreased markedly with depth in the profile. Similar observations regarding the release of ammonium- and amino-acid-N have been made in recent work by Stevenson (1957), but he found that the proportion of soil nitrogen dissolved by acid hydrolysis tended to increase with depth in the profile. This is perhaps due to the fact that in Stevenson's work the soils were hydrolysed with 6N-HCl at about 120° C. for 24 hr. using 5 ml. acid per gramme of soil, whereas in the present work the soils were hydrolysed with 6N-HCl under reflux for 12 hr. using 3 ml. acid per gramme of soil. The latter method was adopted because previous work with surface soils (Bremner, 1949) had shown that maximal release of nitrogen was obtained using 3 ml. acid per gramme of soil and that the amount of  $\alpha$ -amino-N released by hydrolysis decreased if hydrolysis was prolonged beyond about 12 hr. owing to deamination of amino acids. It is possible that more nitrogen could be liberated from subsoils by using a longer period of hydrolysis with a larger volume of 6N-HCl. This possibility clearly deserves attention in future investigations on the forms of nitrogen in soil profile samples using the acid hydrolysis technique. The results in Table 9 suggest that it may also be profitable in such investigations to treat the soil samples with hydrofluoric acid to release ammonium and organic nitrogen compounds held by clay minerals before hydrolysis with 6N-HCl. There seems little doubt, that in previous hydrolysis studies on soil nitrogen, some of the nitrogen classified as non-hydrolysable organic nitrogen was in the form of fixed ammonium. Residues from hydrolysis of lignin-ammonia complexes with 6N-HCl for 12 hr. were found to yield only trace amounts of ammonium when treated with N-HF:N-HCl at room temperature for 24 hr. Calculations from the data in Table 12 show that the proportion of total nitrogen in the form of ammonium was much higher in the subsoil hydrolysates (average, 47%) than in the surface soil hydrolysates (average, 27%).

Even if allowance is made for ammonium derived from fixed ammonium in clay minerals, the amounts of ammonium released by hydrolysis of the surface soils examined are still too large to be satisfactorily accounted for by deamination of nitrogenous materials known to occur in soil. However, when the proportion of organic nitrogen converted to ammonium by acid hydrolysis of surface soils is calculated by making allowance for fixed ammonium, the results are not greatly different from those obtained with practically clay-free humic-acid preparations separated from surface soils (Bremner, 1955).

The amount of material dissolved by hydrolysis of mineral soils with 6N-HCl is considerably less than the amount dissolved by treatment with N-HF:N-HCl. For example, the present work showed that 13.2–18.0% of the material in soils 2, 4 and 6 was dissolved by hydrolysis with 6N-HCl for 12 hr., whereas previous work (Bremner & Harada, 1958) showed that 31.7–33.4% was dissolved by treatment with N-HF:N-HCl at room temperature.

Since previous work (Bremner & Harada, 1958) showed that only a small amount of the nitrogen released on treatment of soil with hydrofluoric acid is extracted by neutral or alkaline reagents, the finding that the proportion of soil nitrogen released by hydrofluoric acid treatment of soil profile samples increased markedly with depth in the profile (Table 2), indicated that the proportion extractable by alkali or neutral pyrophosphate should decrease markedly with depth. This is confirmed by the results in Table 10, which shows the amounts of nitrogen extracted from soil profile samples by a single treatment with alkali or pyrophosphate. Experiments with soils 7a and 7b showed that the proportion of soil nitrogen extracted by repeated treatment with alkali or pyrophosphate also decreased markedly with depth in the profile.

Bal (1925) found that when some Indian soils containing a high percentage of clay were analysed for nitrogen by the Kjeldahl method, the values obtained were considerably higher if the soils were treated with water before analysis. He also noted that the residues from digestion of the soils with sulphuric acid were much coarser and darker in colour when the treatment with water was omitted, and that the effect of this treatment on nitrogen values obtained by the Kjeldahl method was markedly greater with the clay fractions of these soils than with the silt or fine silt fractions. From these and other observations Bal concluded that the soils examined contained some material which cemented the soil particles together and protected organic matter inside the particles from the action of sulphuric acid, and that this cementing material was not readily soluble in concentrated sulphuric acid but was easily dissolved by dilute sulphuric

acid. Several workers (e.g. Srinivasan, 1932; Walkley, 1935; Ashton, 1936) have confirmed Bal's observations regarding the effect of treating clay soils with water before analysis by the Kjeldahl method. Walkley (1935) found that ball-milling of such soils before Kjeldahl analysis had an even greater effect than treatment with water, and that it also led to an increase in values obtained in carbon analysis of the soils. He deduced that the lower nitrogen values obtained with these soils when the treatment with water was omitted were not due to the presence of cementing materials insoluble in concentrated sulphuric acid, but to failure of the soil crumbs to disperse in the non-polar sulphuric acid. These observations suggest that the clay soils examined by Bal and Walkley contained ammonium (and possibly organic) nitrogen within the lattices of clay minerals and that this nitrogen was not determinable by the Kjeldahl method unless the soils were first ball-milled to destroy the clay lattices, or were treated with water to permit expansion of the lattices during treatment with sulphuric acid. This explanation is not supported by the results in Table 11, which show that pretreatment of the soils used in this work with water had no effect on the values obtained by the Kjeldahl method, despite the fact that some of the soils contained large amounts of fixed ammonium and apparently clay-bound organic-nitrogen. However, the period of digestion with sulphuric acid used in these analyses was considerably longer than that generally adopted for soil. It is possible therefore that the lower values obtained with clay soils by Bal and other workers when pretreatment with water was omitted were due to the use of short periods of digestion which did not effect the release of ammonium and organic nitrogen associated with clay minerals. Some support for this explanation was provided by the observation that the residues from digestion of ammonium-saturated vermiculite, and several of the soils with concentrated sulphuric acid for 1 hr. were coarse and dark coloured, whereas the residues after digestion for 5 hr. were as white and finely divided as those from digestion with sulphuric acid for 1 hr. after pretreatment with water. The possibility that the release of fixed ammonium from clay minerals during Kjeldahl digestion of soils is hindered by the large amounts of potassium present was eliminated by experiments which showed that the same results were obtained if sodium sulphate instead of potassium sulphate was used to raise the boiling point of the sulphuric acid. The further possibility that fixed ammonium in clay minerals is not completely released by Kjeldahl digestion with sulphuric acid, but is liberated on distillation of the digests with sodium hydroxide, was also eliminated by experiments which showed that the same amounts of ammonia were liberated

by distillation of Kjeldahl digests of ammonium-saturated vermiculite, and soils 5c, 5d and 7b with potassium hydroxide as by distillation with sodium hydroxide. Taken with results previously obtained in Kjeldahl analysis of clay minerals containing fixed ammonium (Bremner & Harada, 1958), these findings leave little doubt that fixed ammonium in soil is determined quantitatively by the Kjeldahl method, provided an adequate period of digestion with sulphuric acid is employed.

The fact that many soils contain fixed ammonium and can fix added ammonium has many implications besides those already discussed. For example, it means that the ammonium-fixing capacity of soil must be added to the list of factors to be considered in studies on nitrogen transformations in soil, and in studies on the mineralization of nitrogenous materials by soil micro-organisms using the soil-incubation technique. It also suggests that in seeking to account for the ability of some clay minerals to retard the rate of decomposition of nitrogenous organic materials by soil micro-organisms, some attention should be given to the possibility that the stabilizing effect of the minerals may be partly due to their ability to fix ammonium liberated by mineralization of the organic materials. It further suggests that the increase in exchangeable ammonium content, frequently observed when soils are air dried, may be partly due to the fact that on air drying some fixed ammonium is converted to the exchangeable form by a mechanism similar to that responsible for the release of exchangeable potassium from the fixed form when some soils are air dried. The findings that even exfoliated vermiculite can fix significant amounts of ammonium under moist conditions at room temperature (Allison, Roller & Doetsch, 1953) and that small amounts of potassium block the release of fixed ammonium from clay minerals, add to previous criticism (Harmsen & Van Schreven, 1955) of the practice of adding vermiculite or potassium salts to increase the rate of nitrification in soil incubation experiments. The fact that some soils can fix added ammonium provides an explanation of the inability of many workers to recover ammonium added to some soils by methods of extraction found effective with other soils.

The results obtained by the  $N\text{-HF:N-HCl}$  method of determining fixed ammonium in soil would appear to be considerably more reliable than those obtained by the other methods available, but their accuracy cannot be assessed until new methods of determining fixed ammonium are developed. A method based on determination of the amount of ammonium released by fine grinding of soil may be of some value, since grinding under certain conditions may destroy the crystal lattices of clay minerals and release fixed ammonium without causing serious decomposition of organic nitrogen compounds to ammonium. The

results presented indicate that treatment with N-HF:N-HCl at room temperature for 24 hr. using 20 ml. reagent per gramme of soil effects practically quantitative release of fixed ammonium from most soils, but that a longer period of treatment with a larger volume of reagent is sometimes required for complete release. For routine purposes it is suggested that a method based on treatment with N-HF:N-HCl for 24 hr. using 40 ml. reagent per gramme of soil be employed. The inclusion in the method of a pretreatment with KOH is theoretically desirable, since it should reduce the risk of interference by organic nitrogen compounds and render the clay minerals more susceptible to attack by hydrofluoric acid. However, the results so far obtained using this pretreatment (Table 6) do not justify its adoption in routine work.

In conclusion, it should be pointed out that the finding that Rodrigues's method of determining fixed ammonium in soil causes serious decomposition of organic nitrogen compounds to ammonium does not invalidate Rodrigues's general conclusions or seriously detract from the value of his observations. The main conclusion from his work, namely, that a considerable fraction of the nitrogen in some soils is in the form of fixed ammonium, is amply confirmed by the results reported here, and it is apparent that the belief that only about 1-2% of soil nitrogen occurs in inorganic form is no longer tenable. It should also be noted that although Barshad's alkaline distillation method of determining fixed ammonium has been employed for the determination of naturally occurring fixed ammonium in soil, there would appear to be no indications that Barshad intended that the method be used for this purpose.

### SUMMARY

1. A method of determining fixed ammonium in soil based on estimation of the difference in the amounts of ammonium released on treatment with

N-HF:N-HCl and with N-KCl has been investigated and compared with the hydrofluoric acid method of Rodrigues and the alkaline distillation method of Barshad.

2. Results obtained by the N-HF:N-HCl procedure with profile samples of various soils indicated that 3-8% (average, 5.6%) of the nitrogen in the surface soils and 9-44% (average, 21.5%) of the nitrogen in the subsoils examined was in the form of fixed ammonium. Rodrigues's method gave much higher values and Barshad's method gave much lower values.

3. Studies on the forms of nitrogen in soil profiles showed that the proportion of soil nitrogen released by acid hydrolysis as total-N and  $\alpha$ -amino-N decreased with depth in the profile, whereas the proportion liberated as ammonium by acid hydrolysis increased with depth. They also showed that a considerable amount of the ammonium released by acid hydrolysis was derived from fixed ammonium in clay minerals.

4. It was found that the proportion of soil nitrogen dissolved by N-HF:N-HCl increased with depth in the profile, whereas the proportion dissolved by neutral and alkaline reagents used to extract soil organic matter decreased with depth. On the average, 23.2% of the nitrogen in the surface soils and 47.4% of the nitrogen in the subsoils was dissolved by a single treatment with N-HF:N-HCl at room temperature for 24 hr.

5. The reliability and significance of the results obtained by the N-HF:N-HCl method are discussed.

The author wishes to thank Mr G. Brown and Mr C. L. Bascomb for supplying some of the soil samples used in this work. He is also indebted to Mr G. Brown for information regarding the clay mineral composition of some of the samples used.

### REFERENCES

- ALLISON, F. E., ROLLER, E. M. & DOETSCH, J. H. (1953). *Soil Sci.* **75**, 173.
- ALLISON, F. E. & ROLLER, E. M. (1955). *Soil Sci.* **80**, 349.
- ASHTON, F. L. (1936). *J. Agric. Sci.* **26**, 239.
- ATTOE, O. J. (1947). *Proc. Soil Sci. Soc. Amer.* **11**, 145.
- BAL, D. V. (1925). *J. Agric. Sci.* **15**, 454.
- BARSHAD, I. (1948). *Amer. Min.* **33**, 655.
- BARSHAD, I. (1950). *Amer. Min.* **35**, 225.
- BARSHAD, I. (1951). *Soil Sci.* **72**, 361.
- BARSHAD, I. (1954a). *Soil Sci.* **77**, 463.
- BARSHAD, I. (1954b). *Soil Sci.* **78**, 57.
- BREMNER, J. M. (1949). *J. Agric. Sci.* **39**, 183.
- BREMNER, J. M. (1955). *J. Agric. Sci.* **46**, 247.
- BREMNER, J. M. & HARADA, T. (1959). *J. Agric. Sci.* **52**, 137.
- BREMNER, J. M. & SHAW, K. (1954). *J. Agric. Sci.* **44**, 152.
- BREMNER, J. M. & SHAW, K. (1955). *J. Agric. Sci.* **46**, 320.
- BREMNER, J. M. & SHAW, K. (1957). *J. Sci. Fd Agric.* **8**, 341.
- CHAMINADE, R. (1940). *C.R. Acad. Sci., Paris*, **210**, 264.
- HANWAY, J. J. & SCOTT, A. D. (1956). *Soil Sci.* **82**, 379.
- HANWAY, J. J., SCOTT, A. D. & STANFORD, G. (1957). *Proc. Soil Sci. Soc. Amer.* **21**, 29.
- HARMSSEN, G. W. & VAN SCHREVEN, D. A. (1955). *Advanc. Agron.* **7**, 299.
- MARKHAM, R. (1942). *Biochem. J.* **36**, 790.
- MATTSON, S. & KOUTLER-ANDERSSON, E. (1941). *LantbrHögsk. Ann.* **9**, 57.

- MATTSON, S. & KOUTLER-ANDERSSON, E. (1943). *LantbrHögsk. Ann.* **11**, 107.
- NASH, V. E. & MARSHALL, C. E. (1957). *Proc. Soil Sci. Soc. Amer.* **21**, 149.
- NÖMMIK, H. (1957). *Acta agric. Scand.* **7**, 395.
- OLSEN, C. (1929). *C.R. Lab. Carlsberg*, **17**, no. 15.
- RODRIGUES, G. (1954). *J. Soil Sci.* **5**, 264.
- SCOTT, A. D., HANWAY, J. J. & STICKNEY, E. M. (1957). *Proc. Soil Sci. Soc. Amer.* **21**, 499.
- SRINIVASAN, A. (1932). *Indian J. Agric. Sci.* **2**, 525.
- STEVENSON, F. J. (1954). *Proc. Soil Sci. Soc. Amer.* **18**, 373.
- STEVENSON, F. J. (1956). *Proc. Soil Sci. Soc. Amer.* **20**, 204.
- STEVENSON, F. J. (1957). *Proc. Soil Sci. Soc. Amer.* **21**, 283.
- TINSLEY, J. (1950). *Trans. 4th Int. Congr. Soil Sci.* **1**, 161.
- VAN SLYKE, D. D., DILLON, R. T., MACFADYEN, D. A. & HAMILTON, P. (1941). *J. Biol. Chem.* **141**, 627.
- WALKLEY, A. (1935). *J. Agric. Sci.* **25**, 598.

(Received 2 May 1958)

*Note added in proof.*\* Further work has shown that a significant amount of the ammonia-N which is taken up by organic matter when organic soils and lignin preparations are treated with ammonia gas, and which is not extractable by N-KCl, is released (as ammonium-N) by treatment with N-HF:N-HCl at room temperature for 24 hr. The pretreatment with N-KOH described in the paper should therefore be adopted if the N-HF:N-HCl method is used to determine clay-bound ammonium in soils that

have received nitrogen in the form of ammonia gas or ammonium hydroxide. The lignin-ammonia complexes used in the experiments described on p. 156 had been washed thoroughly with 0.5 N-hydrochloric acid to remove adsorbed and exchangeable ammonium. It now seems likely that this acid treatment removed some lignin-bound ammonia-N that would not have been extractable by N-KCl but would have been released (as ammonium) by treatment with N-HF:N-HCl at room temperature.

\* 31 November, 1958