

# RELEASE OF AMMONIUM AND ORGANIC MATTER FROM SOIL BY HYDROFLUORIC ACID AND EFFECT OF HYDROFLUORIC ACID TREATMENT ON EXTRACTION OF SOIL ORGANIC MATTER BY NEUTRAL AND ALKALINE REAGENTS

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One of the main difficulties encountered in work on the nature and properties of soil organic matter is that of separating the organic matter from the mineral constituents of the soil. The classical method of separation using alkali permits isolation of a considerable fraction of the organic matter, but has the disadvantage that the material extracted by alkali is undoubtedly modified in the process. Milder extractants such as sodium fluoride and sodium pyrophosphate are sometimes employed, but they are less efficient than alkaline reagents and for most purposes the alkali extraction technique is preferred. There are grounds for the belief that some of the difficulties encountered in separating the organic and clay fractions of soil are due to their interaction with formation of clay-organic matter complexes. If this is true, it should be possible to increase the efficiency of separation of organic matter by treating the soil with some reagent which decomposes clay minerals but does not seriously modify the organic matter. Hydrofluoric acid shows promise of meeting these requirements, since it has the ability to decompose and dissolve clay minerals and is a weak acid unlikely to cause serious modification of soil organic matter. Rather (1917) used this reagent for the determination of organic matter in soil, his method being based on removal of hydrated clay minerals by repeated treatment with a dilute solution of hydrofluoric and hydrochloric acids before determination of loss on ignition. Alexander & Byers (1932) modified this method by using fewer treatments with a stronger solution of hydrofluoric and hydrochloric acids to dissolve the hydrated silicate minerals, and their modified procedure was employed by Broadbent & Bradford (1952) to obtain soils free of inorganic exchange materials for investigations on the cation-exchange properties of soil organic matter. Gottlieb & Hendricks (1946) adapted Rather's method of dissolving mineral matter to prepare a mineral soil fraction containing a high percentage of organic matter, and separated material containing 32.6 % carbon by HF-HCl treatment of

the fine ( $< 0.1 \mu$ ) fraction of a silt loam. Jones (1948) used hydrofluoric acid to remove clay minerals from soil humic acid preparations.

The primary object of the work described in this paper was to determine if treatment of mineral soils with hydrofluoric acid to remove clay minerals increased the efficiency of extraction of organic matter by neutral and alkaline reagents. A secondary object was to discover if hydrofluoric acid had any promise as a reagent for the release and determination of fixed ammonium nitrogen in soil. A method of determining this form of nitrogen is clearly required, since it is now well established that some soils can fix an appreciable quantity of ammonium in such a way that it is not readily exchangeable or available to plants and micro-organisms (McBeth, 1917; Stanford & Pierre, 1947; Barshad, 1951; Bower, 1951; Allison, Doetsch & Roller, 1951; Allison, Kefauver & Roller, 1953; Hanway & Scott, 1956; Nömmik, 1957). Since present evidence indicates that the ammonium-fixing capacity of soil is due to the presence of clay minerals such as illite, vermiculite and montmorillonite (Barshad, 1948; Allison, Roller & Doetsch, 1953; Allison & Roller, 1955), it should be possible to determine fixed ammonium in soil if some method of releasing fixed ammonium from clay minerals can be found which does not cause significant decomposition of organic forms of soil nitrogen to ammonium. As shown below, the possibilities of a method based on the use of hydrofluoric acid have been investigated.

## MATERIALS AND METHODS

*Soils.* Analytical data concerning the soils used are given in Table 1. Samples 1-4 were from the Broadbalk continuous wheat plots at Rothamsted where the soil is derived from clay-with-flints overlying chalk. These samples were chosen because previous work had shown that only 50-60 % of their nitrogen could be extracted by alkali and that less than 15 % could be extracted by neutral reagents. Sample 1 was from the plot which had received no manure or fertilizer since 1839 (plot 3), sample 2 from a plot receiving complete minerals and nitrogen

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Table 1. *Analysis of soils*

Sample	Depth (in.)	pH	Percentage on moisture-free basis		
			C	N	CaCO <sub>3</sub>
1	0-6	8.2	0.99	0.105	2.1
2	0-6	7.9	1.08	0.117	0.6
3	0-6	7.7	1.09	0.122	0.6
4	0-6	7.8	2.51	0.256	1.4
5b	18-27	7.6	0.37	0.055	0.1
6	0-6	7.5	2.63	0.263	0.2
7b	20-28	5.9	0.14	0.034	0

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). Sample 5b was from a profile of the soil on plot 7 of the Broadbalk field, sample 6 from an arable field on the Weald Clay, and sample 7b from a profile of a brown earth soil in Anglesey. The predominant clay minerals in the samples were of the mica- and kaolinite. Before use the samples were air dried and ground to pass a sixty-mesh sieve. Carbon in the samples was determined by the method of Tinsley (1950), nitrogen by Bal's (1925) modification of the Kjeldahl method, pH by the glass electrode (soil: water ratio, 1:2), and CaCO<sub>3</sub> by the method of Schollenberger (1930).

#### *Analysis of extracts*

Carbon was determined by the method of Tinsley (1950).

Total-N was determined by a micro-Kjeldahl procedure previously described (Bremner, 1949).

Ammonium-N was determined by distillation with MgO at 25° C. in micro-diffusion units (Bremner & Shaw, 1955) or with sodium borate (pH 8.8) buffer (Bremner & Shaw, 1954), extracts containing hydrofluoric acid being neutralized with NaOH before analysis. This neutralization step was introduced because it was found that ammonium-N in ammonium sulphate solutions made 2N with respect to HF, or 1N with respect to both HF and HCl, could not be recovered quantitatively by boiling or steam distilling the solutions with NaOH, MgO or pH 8.8 sodium borate buffer, or by distilling with MgO at 25° C., apparently because some hydrofluoric acid was carried over into the boric acid used to collect the ammonia liberated by distillation. Neutralization (pH 6.3) of the solutions by cautious addition of 2N-NaOH using methyl red as indicator before distillation with alkaline reagents was found to eliminate this difficulty, provided the neutralized solutions were allowed to stand for some time before distillation. In practice it was found more convenient when using the borate buffer method to pipette an aliquot of the soil extract into the Kjeldahl distillation flask, neutralize with NaOH,

add the borate buffer solution, and stopper and shake the flask for a few minutes before attachment to the distillation apparatus. Tests showed that the borate buffer and micro-diffusion methods gave practically identical results with N-HF:N-HCl extracts of soils 1, 4 and 6, and that ammonium-N added to these extracts was recovered quantitatively by both methods. It was also found that ammonium-N in ammonium sulphate solutions made 1N with respect to both HF and HCl and ammonium-N added to N-HF:N-HCl extracts of the soils could be determined quantitatively by the micro-diffusion method without neutralization of the acid with NaOH, provided the aliquots taken for analysis were diluted with an equal volume of water before distillation with MgO and the period of distillation was increased about 50% beyond the time previously recommended (Bremner & Shaw, 1955).

## RESULTS

### *Treatment of soils with hydrofluoric acid*

In Rather's (1917) method for the determination of organic matter in soil, the minerals which interfere with the loss on ignition method were removed by repeated treatment of the soil with a solution approximately 0.25N with respect to HF and 0.14N with respect to HCl, a total of six treatments at 85° C. or on a boiling water-bath being performed. In the method used by Alexander & Byers (1932), the interfering minerals were removed by two overnight treatments at room temperature with a solution approximately 1.25N with respect to HF and 0.68N with respect to HCl. Since the latter method of removing minerals appeared the more convenient, it was decided to use it for a preliminary study of the effect of pretreatment with hydrofluoric acid on the extraction of soil organic matter by alkali using Rothamsted soils. The soils were leached with 0.1N-HCl before use, because previous work had shown that the amount of organic matter extracted from these soils by alkali was increased substantially if the soils were first leached with dilute hydrochloric acid to remove exchangeable metallic cations (Bremner & Lees, 1949). This pretreatment ensured that any effect on extraction with alkali observed as a result of treatment with HF-HCl solution was not merely due to the removal of cations by the HF-HCl solution; it also decomposed carbonates which would otherwise react with the acid used in the HF-HCl treatment. The following procedure was adopted. Acid-leached samples of soils 1-4 were shaken overnight in polythene bottles with 1.25N-HF:0.7N-HCl solution (10 ml./g. of soil) and the mixtures were then centrifuged and the supernatant liquids decanted. This process was repeated and the soil residues were washed with water on the centrifuge and extracted with 0.5N-NaOH (10 ml./g. of

soil), a total of three overnight extractions at room temperature being performed. The alkali extracts were then combined and analysed for nitrogen and carbon. Control experiments in which the soils were not treated with HF-HCl solution before extraction with alkali were also performed. The results of this preliminary work showed that the amounts of nitrogen and carbon extracted from these soils by alkali were not significantly affected by pretreating the soils with HF-HCl solution. For example, extraction of soil 1 with 0.5N-NaOH before treatment with HF-HCl solution dissolved 51.4% of the total soil N, and similar extraction after HF-HCl treatment dissolved 50.5%. Since analysis of the HF-HCl extracts of the soils showed that they contained substantial quantities of nitrogen (18-30% of total soil N), experiments were carried out to discover if the release of nitrogen on treatment of soil with HF-HCl solution was due to the hydrofluoric or the hydrochloric acid. Soil 4 was used, the procedure described above being modified in that three treatments with hydrofluoric or hydrochloric acid solution were performed instead of two treatments with HF-HCl solution. The results (Table 2) showed that treatment of soil with solutions of hydrofluoric acid dissolved considerably more nitrogen than did

treatment with hydrochloric acid solutions of the same strength, and that neither treatment significantly affected the amount of soil nitrogen extracted by alkali.

Since hydrofluoric acid is a much weaker acid than hydrochloric acid and is therefore less liable to cause hydrolysis and decomposition of acid-insoluble soil organic nitrogen compounds with formation of acid-soluble products, these results suggested that treatment of mineral soils with hydrofluoric acid released ammonium and possibly organic nitrogen compounds held by clay minerals. To investigate this possibility, samples of surface soils and subsoils were treated with hydrofluoric and hydrochloric acid solutions for 24 hr., and the amounts of total-N and ammonium-N released by the treatments were determined. The subsoil samples were included because Rodrigues (1954) found that the proportion of soil nitrogen apparently in the form of fixed ammonium increased markedly with depth in some tropical soil profiles. The results (Table 3) showed that treatment of the soils with hydrofluoric acid released considerably more ammonium-N and organic-N (calculated from total-N minus ammonium-N) than did treatment with hydrochloric acid, and that a large proportion of the nitrogen in the two subsoil samples examined was released as ammonium-N by treatment with hydrofluoric acid.

Table 2. Amounts of nitrogen dissolved by treatment of soil with acids and by extraction with sodium hydroxide after acid treatment

(Soil used (no. 4) had been previously leached with 0.1N-HCl. Direct extraction of this leached soil with 0.5N-NaOH dissolved 60.6% of the total soil N.)

Acid	N dissolved (% total soil N)		
	Acid	0.5N-NaOH	Total
HCl, 0.3N	2.4	60.0	62.4
0.6N	3.2	59.8	63.0
1.0N	4.3	60.6	64.9
2.0N	6.7	60.0	66.7
HF, 0.3N	6.7	58.8	65.5
0.6N	10.3	59.4	69.7
1.0N	13.3	59.9	73.2
2.0N	16.0	58.7	74.7

*Release of fixed ammonium from clay minerals and soils by hydrofluoric acid*

The release of fixed ammonium by hydrofluoric acid and other reagents was studied using samples of minerals and soils which had been ammonium-saturated with normal ammonium chloride solution, leached with 95% ethanol until ammonium could not be detected in the leachate by Nessler's reagent, and dried. The following minerals were used: vermiculite from Dupré Vermiculite (Exfoliators) Ltd. (unexfoliated 'Veri-Gro' ore); hydrobiotite (mixed-layer mica-vermiculite structure) from Fukushima, Japan; illite (sample I, from Mr G. Brown; sample II (Fithian illite), from Dr F. J.

Table 3. Amounts of total-N and ammonium-N released by treatment of soils with acids

(Soils were shaken with acids (20 ml. acid/g. of soil) at room temperature for 24 hr.)

Soil ...	Nitrogen released (% total soil N)							
	2		4		5b		7b	
	Total	Ammonium	Total	Ammonium	Total	Ammonium	Total	Ammonium
HCl, 0.5N	3.1	1.2	2.8	1.2	—	1.2	—	2.8
1.0N	4.8	2.2	4.3	1.6	—	3.0	10.2	4.2
2.0N	10.3	3.3	6.8	2.0	21.9	3.7	—	7.0
HF, 0.5N	18.4	4.4	10.1	2.4	—	7.2	—	22.1
1.0N	24.8	6.4	11.2	2.9	—	16.8	46.9	37.9
2.0N	27.1	7.3	13.4	3.9	41.1	19.1	48.1	39.0
N-HF:N-HCl	28.5	8.0	15.9	4.4	41.9	19.8	57.8	47.9

Stevenson); bentonite (sample I, from Matsuida, Gumma, Japan; sample II (Wyoming bentonite), from Dr R. Greene-Kelly); partially decomposed granite from Morioka, Iwate, Japan, which contained hydrobiotite. Various British and Japanese soils known to contain vermiculite, illite or montmorillonite were used, but only one sample was found to fix a significant quantity of ammonium against extraction with *N*-KCl. This sample was from the 6–12 in. layer of a profile in Yahatahama, Ehime, Japan, where the soil is derived from crystalline schist. It contained 13 % clay with vermiculite as the predominant clay mineral. Studies on the release of fixed ammonium in these ammonium-saturated materials by various reagents showed that solutions of hydrochloric acid were considerably more effective than neutral salt solutions such as *N*-NaCl and *N*-CaCl<sub>2</sub>, but much less effective than solutions of hydrofluoric acid. For example, treatment of the ammonium-saturated hydrobiotite with *N*-HCl at room temperature for 24 hr. released about 40 % of the fixed ammonium, whereas similar treatment with *N*-HF released more than 90 %. The amount of fixed ammonium released by treatment of the materials with hydrofluoric acid was found to

increase with the period of treatment and with the volume and strength of acid used, but complete release of fixed ammonium could not be achieved with this reagent under the conditions tested, i.e. by treatment at room temperature with solutions up to 2*N* with respect to hydrofluoric acid for periods up to 24 hr. However, it was found that fixed ammonium was released quantitatively from all the materials studied by treatment at room temperature for 24 hr. with *N*-HF solution made 1*N* with respect to HCl. The results obtained with this reagent are given in Table 4. Total-N in the materials was determined by the Kjeldahl method, a 5 hr. period of digestion with sulphuric acid and K<sub>2</sub>SO<sub>4</sub>:CuSO<sub>4</sub>.5H<sub>2</sub>O:Se (10:1:0.1) mixture being used. Ammonium-N released by treatment with *N*-KCl and with *N*-HF:*N*-HCl was determined by distillation with sodium borate buffer (pH 8.8). The vermiculite and hydrobiotite samples were treated with twice the volume of *N*-HF:*N*-HCl solution used with the other materials; only 92–95 % of the fixed ammonium in these samples was released by treatment with *N*-HF:*N*-HCl for 24 hr. using 20 ml. of reagent per gramme of sample.

The recovery of fixed ammonium by the *N*-HF:*N*-

Table 4. Total-N contents of ammonium-fixing materials and amounts of ammonium-N released from materials by treatment with *N*-HF:*N*-HCl and with *N*-KCl before and after saturation with ammonium

(A, Ammonium-saturated material; B, original material; C, amount in ammonium-saturated material minus corresponding amount in original material.)

Material		M-equiv./100 g. of material		
		Total-N	NH <sub>4</sub> -N released	
			<i>N</i> -HF: <i>N</i> -HCl*	<i>N</i> -KCl†
Vermiculite	A	94.9	94.5	11.9
	B	0	0	0
	C	94.9	94.5	11.9
Hydrobiotite	A	27.9	27.9	7.0
	B	4.3	4.3	0
	C	23.6	23.6	7.0
Bentonite I	A	51.4	51.3	47.2
	B	0.2	0.2	0.1
	C	51.2	51.1	47.1
Bentonite II	A	85.3	85.2	75.3
	B	0.3	0.2	0.1
	C	85.0	85.0	75.2
Illite I	A	20.3	20.3	15.9
	B	4.0	4.0	0.1
	C	16.3	16.3	15.8
Illite II	A	31.4	29.8	21.3
	B	8.8	7.2	0.3
	C	22.6	22.6	21.0
Decomposed granite	A	11.1	11.1	2.9
	B	0	0	0
	C	11.1	11.1	2.9
Yahatahama soil	A	37.0	32.2	12.3
	B	5.1	0.3	0.1
	C	31.9	31.9	12.2

\* Materials were shaken with *N*-HF:*N*-HCl (40 ml./g. of vermiculite or hydrobiotite; 20 ml./g. of other materials) at room temperature for 24 hr.

† Materials were shaken with *N*-KCl (40 ml./g. of material) at room temperature for 24 hr.

Table 5. Amounts of ammonium-N released on treatment of various nitrogenous organic materials with neutral and acid reagents

(Materials were treated with reagents at 25° C. for 24 hr.)

Reagent Material	Ammonium-N released (% total N in material)			
	N-KCl	N-HF	N-HCl	N-HF:N-HCl
Edestin	0.1	0.5	0.9	0.9
Casein	0.2	0.6	1.0	1.1
Gelatine	0	0.1	0.2	0.2
Alanine	0	—	—	0
Serine	0	—	—	0
Asparagine	0.2	0.8	3.2	3.6
Glutamine	4.4	10.9	20.9	21.6
Allantoin	0	0	0	0
Glucosamine	0.6	—	1.2	1.4
Chitin	0.3	—	—	0.7
Humic acid I	0.1	0.4	—	0.8
Humic acid II	0.1	0.5	—	0.9

Table 6. Amounts of total-N and ammonium-N released on treatment of organic soils with neutral and acid reagents

(Soils were treated with reagents at room temperature for 24 hr.)

Soil	Reagent	N released (% total soil N)	
		Total	Ammonium
Mountain peat	N-KCl	6.0	3.0
	N-HF	6.0	3.0
	N-HCl	7.8	3.1
	N-HF:N-HCl	7.9	3.2
Low-moor peat	N-KCl	5.3	1.0
	N-HF	5.5	1.2
	N-HCl	5.9	1.5
	N-HF:N-HCl	6.1	1.7
Sedge peat	N-KCl	4.2	1.3
	N-HF	4.3	1.4
	N-HCl	4.7	1.7
	N-HF:N-HCl	4.9	1.9

HCl method was also studied by treating 2 g. samples of the materials which fixed ammonium under moist conditions at room temperature (vermiculite, hydrobiotite, Yahatahama soil, and partially decomposed granite) with 2 ml. of water or of ammonium chloride solution containing 7.0 mg. of ammonium-N for 24 hr., and then analysing for total-N (Kjeldahl method) and for ammonium-N released by treatment with N-KCl and with N-HF:N-HCl. The Kjeldahl analyses were performed by treating the moist samples with 10 g. K<sub>2</sub>SO<sub>4</sub>, 1 g. CuSO<sub>4</sub>.5H<sub>2</sub>O, 0.1 g. Se, and 40 ml. conc. H<sub>2</sub>SO<sub>4</sub> and boiling the mixtures gently for 5 hr., ammonium-N in the digests being determined by distillation with NaOH. The N-KCl and N-HF:N-HCl treatments were performed by treating the moist samples with 38 ml. water and either 40 ml. 2N-KCl or 40 ml. 2N-HF:2N-HCl and shaking the mixtures for 24 hr.; ammonium-N released was determined

by distillation with borate buffer (pH 8.8). The results showed that 0.74–5.01 mg. of the 7 mg. of ammonium-N added to these materials was recovered by extraction with N-KCl and that 6.93–7.05 mg. was recovered by Kjeldahl digestion or by treatment with N-HF:N-HCl.

*Release of ammonium on treatment of nitrogenous organic materials with hydrofluoric and hydrochloric acid*

Table 5 shows the amounts of ammonium-N released on treatment of various nitrogenous organic materials with N-KCl, N-HF, N-HCl and N-HF:N-HCl at 25° C. for 24 hr. The treatments were performed with occasional shaking in polythene bottles (50 ml. reagent/weight of material containing 25 mg. N), a few drops of toluene being added to inhibit microbial action. Ammonium-N released by the treatments was determined by distillation with MgO at 25° C. The treatments with N-KCl were performed as controls to allow for ammonium-N present in the materials before treatment with acid and that formed by decomposition of the materials under the conditions used to determine ammonium-N. The humic acids employed were preparations isolated by acidification (pH 2.0) of neutral sodium pyrophosphate extracts of fen soils. Further information concerning these materials is given in a previous paper (Bremner, 1955) in which they are referred to as preparations 1P (humic acid I) and 3P (humic acid II).

*Release of ammonium on treatment of organic soils with hydrofluoric and hydrochloric acid*

Table 6 shows the amounts of ammonium-N and total-N released on treatment of three organic soils with N-KCl, N-HF, N-HCl and N-HF:N-HCl at room temperature for 24 hr. The organic soils used have already been described (Bremner, 1955). Determin-

nations of loss on ignition (500° C.) of the soils gave the following results: low-moor peat (2.82% N), 19.8%; mountain peat (2.07% N), 5.5%; sedge peat (2.46% N), 14.6%. Before use the soils were ground to pass a 100-mesh sieve. The treatments were performed with continuous shaking in polythene bottles (40 ml. reagent/g. of soil), a few drops of toluene and capryl alcohol being added to inhibit microbial action and promote wetting of the soils by the reagents. Ammonium-N released by the treatments was determined by distillation with MgO at 25° C.

*Estimation of fixed ammonium in mineral soils by N-HF:N-HCl method*

The findings reported in Tables 4–6 indicated that treatment of mineral soils with N-HF:N-HCl at room temperature for 24 hr. should effect quantitative release of fixed ammonium in clay minerals without causing significant decomposition of organically bound nitrogen to ammonium-N, and that fixed ammonium-N should therefore be estimable from the difference in the amounts of ammonium-N released by treatment with N-HF:N-HCl and with N-KCl. The results obtained by this method with the soils used in this work are given in Table 7, which also shows the amounts of ammonium-N and total-N released by treatment of the soils with 0.1N-HCl and by N-HF:N-HCl treatment after treatment with 0.1N-HCl. In the treatments with 0.1N-HCl and N-KCl, 30 g. samples of the soils were shaken with 250 ml. reagent for 2 hr., the mixtures were filtered on sinter-glass funnels, and the residues were washed with fresh reagent until 500 ml. of filtrate had been collected. In the treatment with N-HF:N-HCl, 10 g. samples of the soils were shaken with 200 ml. reagent at room temperature for 24 hr. The results in Table 4 indicate that this treatment should effect complete release of fixed ammonium from

soils containing up to 50% clay, even if the clay is entirely in the form of vermiculite or hydrobiotite. None of the soils examined contained more than 45% clay. It can be seen from Table 7 that the proportion of soil nitrogen in the form of fixed ammonium, as estimated by the N-HF:N-HCl method, was considerably higher in the subsoils (18.6–44.5%) than in the surface soils (4.0–7.8%), and that pretreatment of the soils with 0.1N-HCl had little effect on the release of ammonium-N by N-HF:N-HCl.

*Effect of pretreatment with N-HF:N-HCl on extraction of nitrogen from soil by alkaline and neutral reagents*

Table 8 shows the amounts of total- and ammonium-N released by treatment of the soils with N-HF:N-HCl, the effect of this treatment on extraction of nitrogen by 0.5N-sodium hydroxide, 0.5M-sodium fluoride and 0.1M-sodium potassium pyrophosphate (pH 7.0), and the total amounts of nitrogen dissolved by treatment with N-HF:N-HCl followed by extraction with 0.5N-sodium hydroxide. The soils used in these experiments had been previously leached with 0.1N-HCl. The procedure employed was similar to that used in the work reported in Table 2, the acid-leached soils being treated four times overnight with N-HF:N-HCl (10 ml./g. of soil) and three times overnight with the alkaline or neutral reagent (10 ml./g. of soil). The neutral pyrophosphate solution was prepared by mixing 0.1M-Na<sub>2</sub>H<sub>2</sub>P<sub>2</sub>O<sub>7</sub> with 0.1M-K<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. The results show that treatment of the soils with N-HF:N-HCl had little effect on the extraction of nitrogen by alkaline or neutral reagents, and that over 80% of the nitrogen in the soils was dissolved by treatment with N-HF:N-HCl followed by extraction with 0.5N-NaOH. Further work showed that more than 90% of the nitrogen in soils 1–4 was dissolved if further treatments with N-HF:N-HCl and

Table 7. Amounts of total-N and ammonium-N released by treatment of soils with 0.1N-HCl, N-KCl and N-HF:N-HCl and amounts of fixed ammonium-N in soils as determined by N-HF:N-HCl method

(A, N released by N-HF:N-HCl before treatment of soil with 0.1N-HCl; B, N released by N-HF:N-HCl after treatment with 0.1N-HCl.)

Soil	N released (% total soil N)									Fixed NH <sub>4</sub> -N† (% total soil N)
	0.1N-HCl		N-KCl		N-HF:N-HCl					
	Total	NH <sub>4</sub>	Total	NH <sub>4</sub>	A		B			
					Total*	NH <sub>4</sub> *	Total*	NH <sub>4</sub> *		
1	0.9	0.4	0.7	0.3	24.1	6.4	23.9	6.3	6.1	
2	1.3	0.7	1.0	0.5	28.9	8.3	27.9	7.7	7.8	
3	1.2	0.4	0.6	0.2	26.4	7.5	25.6	7.3	7.3	
4	1.1	0.7	0.9	0.4	16.1	4.4	15.5	4.0	4.0	
5b	—	1.1	—	0.9	41.5	19.5	—	—	18.6	
6	1.1	0.6	0.8	0.4	19.6	6.0	19.4	5.9	5.6	
7b	—	2.5	—	2.3	56.7	46.8	—	45.6	44.5	

\* Calculated as percentage of total N in soil after treatment with 0.1N-HCl.

† Ammonium-N released by N-HF:N-HCl minus ammonium-N released by N-KCl.

0.5N-NaOH were carried out. This result was confirmed by Kjeldahl analysis of the residues from the treatments.

*Effect of pretreatment with neutral reagents on release of nitrogen from soils by N-HF:N-HCl*

Table 9 shows the amounts of total- and ammonium-N released by treatment of soils with N-HF:N-HCl before and after extraction with 0.5M-sodium fluoride or neutral 0.1M-sodium potassium pyrophosphate. The soils used had been previously leached with 0.1N-HCl. The extractions with sodium fluoride and pyrophosphate were carried out as described in the previous section. Only one treatment with N-HF:N-HCl was performed, the soils being shaken with the reagent (20 ml./g. of soil) at room temperature for 24 hr. It can be seen that the amounts of total- and ammonium-N released by N-HF:N-HCl were not significantly affected if the soils were first extracted with sodium fluoride or pyrophosphate.

*Fractionation of carbon and nitrogen by treatment of soils with N-HF:N-HCl*

In view of the difficulties associated with the determination of carbon in HF-HCl extracts of

soil, the amounts of carbon dissolved on treatment of soils with N-HF:N-HCl were determined by analysis of the residues from this treatment. The procedure adopted was as follows. Samples of soils 2, 4 and 6 were shaken in polythene bottles with N-HF:N-HCl (10 ml./g. of soil) for 24 hr., and the mixtures were then centrifuged and the supernatant liquids decanted. This process was twice repeated and the soil residues were washed with water on the centrifuge, dried to constant weight *in vacuo* over KOH at 25° C., and analysed for carbon and nitrogen. The amounts of total- and ammonium-N released by HF-HCl treatment of the soils were also determined. The results are given in Table 10, which shows the amounts of carbon and total-N in the HF-HCl extracts of the soils as calculated from the amounts present in the residues from the HF-HCl treatment. The amounts of total-N actually found in the extracts (soil 2, 30.4 % of total soil N; soil 4, 20.2 %; soil 6, 23.8 %) were practically identical to the calculated values. The results show that the HF-HCl treatment dissolved about one-third of the material in the soils and released a considerable amount of carbon as well as nitrogen. Treatment of soil 6 with N-HCl instead of with N-HF:N-HCl as described above was found to

Table 8. Amounts of nitrogen extracted from soils by 0.5N-sodium hydroxide, 0.1M-sodium potassium pyrophosphate (pH 7.0) and 0.5M-sodium fluoride before and after treatment of soils with N-HF:N-HCl

(Soils used had been previously leached with 0.1N-HCl. A, N dissolved by reagent before treatment of soil with N-HF:N-HCl; B, N dissolved by reagent after treatment with N-HF:N-HCl.)

Soil	N-HF:N-HCl*	N extracted (% total soil N)							
		0.5N-NaOH		0.1M-NaK pyrophosphate		0.5M-NaF		N-HF:N-HCl and 0.5N-NaOH	
		A	B	A	B	A	B		
1	31.4 (7.0)	51.4	50.0	12.6	10.7	10.8	10.0	81.4	
2	32.1 (8.1)	53.6	51.0	13.4	12.7	11.2	10.9	83.1	
3	31.9 (8.0)	52.8	51.2	13.0	11.9	11.5	11.1	83.1	
4	21.9 (4.8)	60.3	59.8	11.9	11.1	10.2	11.0	81.7	
6	25.3 (6.2)	60.2	58.2	15.7	12.4	10.7	9.0	83.5	

\* Figures in parentheses represent percentages of total soil N released as ammonium-N by treatment with N-HF:N-HCl.

Table 9. Amounts of total-N and ammonium-N released by treatment of soils with N-HF:N-HCl before and after extraction with 0.5M-sodium fluoride and 0.1M-sodium potassium pyrophosphate (pH 7.0)

(Soils used had been previously leached with 0.1N-HCl.)

Soil	...	...	...	...	N released by N-HF:N-HCl (% total soil N)					
					2		4		6	
					Total	Ammonium	Total	Ammonium	Total	Ammonium
Before extraction with fluoride or pyrophosphate					27.2	7.5	15.6	4.1	19.1	5.5
After extraction with 0.5M-sodium fluoride					28.1	8.2	16.0	4.3	18.4	5.1
After extraction with 0.1M-NaK pyrophosphate					26.2	7.1	16.8	4.9	20.1	6.0

Table 10. Fractionation of carbon and nitrogen by treatment of soils with N-HF:N-HCl

	Soil no. ... ..	2	4	6	
Soil	C content (%)	1.08	2.51	2.63	
	N content (%)	0.117	0.256	0.263	
	C/N ratio	9.2	9.8	10.0	
Residue from HF-HCl treatment	C content (%)	1.19	3.13	3.19	
	N content (%)	0.120	0.304	0.304	
	C/N ratio	9.9	10.3	10.5	
	Amount (% by weight of soil)	68.3	66.6	66.6	
	Residue-C as % soil C	75.2	83.0	80.8	
	Residue-N as % soil N	70.0	79.1	77.0	
HF-HCl extract	Extract-C as % soil C	24.8	17.0	19.2	
	Extract-N as % soil N	Total	30.0	20.9	23.0
		Ammonium	8.4	4.7	6.0
		Organic	21.6	16.2	17.0
	C/total N ratio	7.6	8.0	8.4	
	C/organic N ratio	10.6	10.2	11.3	

dissolve 3.8% of the material in the soil and to release 6.3% of the total soil nitrogen as organic-N and 1.2% as ammonium-N.

#### DISCUSSION

The results given in Tables 2, 3, 8 and 10 show that treatment of the mineral soils used in this work with hydrofluoric acid to decompose clay minerals released considerable amounts of ammonium and organic matter, but did not significantly affect the amounts of organic material extractable from the soils by alkaline and neutral reagents used for the separation of soil organic matter. Taken with the finding that hydrofluoric acid releases fixed ammonium from clay minerals (Table 4), these results indicate that the soils examined contained ammonium and organic matter intimately associated with clay minerals and that this clay-bound material was not extracted by neutral or alkaline reagents, but was released and dissolved when the clay minerals were decomposed with hydrofluoric acid. This deduction is supported by the finding that the amounts of ammonium- and organic-N released by treatment of the soils with N-HF:N-HCl were not significantly affected if the soils were first extracted with sodium fluoride or pyrophosphate (Table 9). The indications from results in Table 8 that pretreatment of soil with hydrofluoric acid may decrease the amount of soil nitrogen extracted by alkaline or neutral reagents have been confirmed in recent work with other soils.

The findings reported in Tables 4-6 suggest that fixed ammonium in soil should be estimable from the difference between the amount of ammonium released by treatment with N-HF:N-HCl for 24 hr. and the amount extracted by N-KCl. The results obtained by this method (Table 7) showed that the proportion of soil nitrogen in the form of fixed ammonium was much higher in the subsoils than in the surface soils examined (cf. Rodrigues, 1954).

The possibility that the treatment with N-HF:N-HCl used in this method causes significant decomposition of organically bound nitrogen in mineral soils to ammonium-nitrogen cannot be tested directly. However, the findings that only 0.2-0.7% of the nitrogen in organic soils and that less than 1% of the nitrogen in various nitrogenous materials other than glutamine and asparagine was converted to ammonium-N by this treatment (Tables 5 and 6), indicate that the results obtained by this method are not seriously affected by decomposition of organic nitrogen compounds. Acid-labile low-molecular nitrogenous organic compounds such as glutamine and asparagine are not likely to occur in soil in more than trace amounts and interference by such compounds should therefore be negligible. This is supported by the results in Table 7, which show that pretreatment of soil with 0.1N-HCl (which should remove compounds such as glutamine and asparagine) had little effect on the results obtained by the N-HF:N-HCl method. The possibility that treatment of mineral soils with N-HF:N-HCl will cause significant decomposition of organically bound nitrogen to ammonium-N is decreased by the fact that a considerable amount of the acid used in this treatment is neutralized by reaction with clay minerals.

Comparison of the results in Tables 6 and 7 shows that, calculated as a percentage of total soil nitrogen, the amount of organic nitrogen released by N-HF:N-HCl in excess of the amount released by N-KCl was much smaller with organic soils (0.1-1.7%) than with mineral soils (11.2-20.1%).

The findings that considerable amounts of organic matter are released by treatment of mineral soils with hydrofluoric acid, and that over 90% of the nitrogen in such soils can be dissolved by repeated treatments with hydrofluoric acid and alkali, do not help greatly towards solution of the problem of finding better methods of separating soil organic matter. The treatment with hydrofluoric acid dis-



solves a large quantity of inorganic material (Table 10), which must be removed before the organic matter dissolved can be characterized satisfactorily, and it may cause some hydrolysis and modification of the organic material even though the indications are that it does not convert significant amounts of organic-N to ammonium-N. However, it may ultimately prove necessary to employ hydrofluoric acid to release clay-bound organic material, since the only apparent alternative method of releasing this material is to ball-mill or otherwise grind the soil until the crystal lattices of clay minerals are destroyed, and the value of this method has not so far been investigated.

Rather (1917) and Alexander & Byers (1932) did not determine the amounts of nitrogen dissolved by the hydrofluoric acid treatments they used to remove hydrated soil minerals, but they did estimate the amounts of carbon dissolved from analysis of residues from the treatments. Calculations from their data show that, on the average, the treatment used by Rather dissolved about 4% of the carbon in the soils examined, while the treatment used by Alexander & Byers dissolved about 23% of the soil organic matter. The results obtained in the present work (Table 10) showed that repeated treatment with N-HF:N-HCl dissolved 17–25% of the carbon and 21–30% of the nitrogen in three of the surface soils examined.

Choudhri & Stevenson (1957) have recently studied the effects of certain treatments, including treatment with hydrofluoric acid to dissolve silicate minerals, on the extraction of nitrogen from soil by alkali and pyrophosphate. They found that the amount of nitrogen extracted by alkali after repeated treatment of soil with 0.3N-HF:0.1N-HCl solution was slightly smaller than the amount extracted by alkali after pretreatment with 0.1N-HCl, but that the amount of nitrogen (particularly humic nitrogen) extracted by neutral sodium pyrophosphate was increased significantly by the HF-HCl treatment. They also observed that the HF-HCl treatment dissolved a considerable amount (up to 18%) of the nitrogen in the soils examined and

postulated that some of this nitrogen was derived from fixed ammonium.

### SUMMARY

1. The effect of treating mineral soils with hydrofluoric acid to decompose clay minerals on the extraction of soil organic matter by alkaline and neutral reagents has been investigated.

2. It was found that treatment of soil with hydrofluoric acid had little effect on the extraction of organic matter by alkaline or neutral reagents, but that it released considerable amounts of ammonium and organic matter. More than 90% of the nitrogen in Rothamsted soils was dissolved by repeated treatments with dilute solutions of hydrofluoric acid and sodium hydroxide.

3. It is shown that treatment of clay minerals with N-HF:N-HCl solution at room temperature for 24 hr. effects quantitative release of fixed ammonium from clay minerals and that similar treatment of soil is not likely to cause significant decomposition of organic nitrogen compounds to ammonium. It is suggested that a method based on this treatment may prove useful for the determination of fixed ammonium in soil. Results obtained by this method indicated that 4–8% of the nitrogen in surface soils and 19–45% of the nitrogen in subsoils examined was in the form of fixed ammonium.

4. It is concluded that some mineral soils contain a significant quantity of ammonium and organic matter intimately associated with clay minerals and that this clay-bound material is not dissolved by neutral and alkaline reagents used for the extraction of soil organic matter, but is released by hydrofluoric acid.

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