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Determination and Isotope-Ratio Analysis of Different Forms of Nitrogen in Soils: 3. Exchangeable Ammonium, Nitrate, and Nitrite by Extraction-Distillation Methods[1]

J. M. Bremner and D. R. Keeney[2]

ABSTRACT

Methods of determining exchangeable ammonium, nitrate, and nitrite in soils are described. They involve extraction of the soil sample with 2M KCl (10 ml/g of soil) and analysis of the extract by steam-distillation methods in which magnesium oxide is used for distillation of ammonium, ball-milled Devarda alloy for reduction of nitrate and nitrite to ammonium, and sulfamic acid for destruction of nitrite. The distillation methods are rapid, accurate, and precise, have high specificity, and are applicable to turbid, colored, and unfiltered soil extracts. They give quantitative recovery of ammonium, nitrate, and nitrite added to soil extracts and permit nitrogen isotope-ratio analysis of these forms of nitrogen in N^{15}-tracer studies of nitrogen transformations in soils.

The methods currently favored for determination of exchangeable ammonium, nitrate, and nitrite in soils involve extraction of these forms of nitrogen and analysis of the extract by colorimetric or distillation techniques. The defects of these methods have been discussed in a recent monograph (2), and it suffices here to say that most of them have serious limitations. For example, the extraction procedures commonly used for determination of nitrate or nitrite by colorimetric methods are not satisfactory for determination of exchangeable ammonium, which means that at least two extractions must be performed when analyses for exchangeable ammonium, nitrate, and nitrite are required. Also, some of the extraction procedures used lead to biological or nonbiological reactions resulting in an increase or decrease in the amount of the form of nitrogen under analysis or yield extracts which are unstable and cannot be safely stored before analysis. The main defects of the methods generally used for determination of inorganic forms of nitrogen in soil extracts are that they lack sensitivity, are subject to interference by organic or inorganic soil constituents, are inapplicable to turbid or colored extracts, or are tedious and time-consuming.

Development of a method for determination of exchangeable ammonium in soils is complicated by lack of generally accepted definitions of exchangeable ammonium and fixed ammonium. The Soil Science Society of America (9) has recommended definition of ammonium fixation as "the adsorption or absorption of ammonium ions by the mineral or organic fractions of the soil in a manner that they are relatively insoluble in water and relatively unexchangeable by the usual methods of cation exchange", but it has not proposed definitions of fixed or exchangeable ammonium. However, most workers have found it convenient to define fixed (nonexchangeable) ammonium as ammonium which is not extractable by 1M KCl at laboratory temperatures, and, since this definition has gained considerable acceptance, it seems logical to define exchangeable ammonium as ammonium which is extractable by 1M KCl at room temperature. In the methods of analysis described here, exchangeable ammonium in soils is arbitrarily defined as ammonium extractable by 2M KCl at room temperature, because it has been found that use of 2M KCl has practical advantages and that the amount of ammonium extracted from soils by 2M KCl is identical to the amount extracted by 1M KCl.

The objective in the work reported was to develop methods of determining exchangeable ammonium, nitrate, and nitrite in soils in which only one extraction of the soil sample is required for performance of these analyses and the extract can be stored for some time before analysis. Additional requirements were that the methods should be rapid, specific, and precise and should permit nitrogen isotope-ratio analysis of exchangeable ammonium, nitrate, and nitrite in N^{15}-tracer studies of nitrogen transformations in soils. The methods described have been found to meet these requirements. They involve extraction of the soil sample by shaking it with 2M KCl (10 ml/g of soil) for 1 hr and analysis of the extract by steam-distillation methods in which the form of nitrogen under analysis is converted to, and estimated as, ammonium, which is readily oxidized to nitrogen gas for mass-spectrometer assay of N^{15} (8). The distillation techniques employed have been described (4). They involve use of ignited magnesium oxide for distillation of ammonium, finely-divided Devarda alloy for reduction of nitrate and nitrite to ammonium, and sulfamic acid for destruction of nitrite ($NH_2SO_3H + HNO_2 = N_2 + H_2SO_4 + H_2O$). They are rapid, accurate, and precise, have high specificity, and are applicable to turbid, colored, and unfiltered soil extracts.

MATERIALS

Soils—The soils used (Table 1) were surface (0- to 15-cm) samples previously air-dried and crushed to pass a 2-mm

Table 1—Analyses of soils

Soil No.	Soil Type	pH	Organic carbon	Total nitrogen	Cation-exchange capacity
			%	%	meq/100 g
1	Nicollet l	6.9	1.53	0.168	20.5
2	Houston sic	7.9	2.33	0.195	41.8
3	Marshall sicl	4.8	2.12	0.209	26.8
4	Webster cl	6.6	3.19	0.244	40.2
5	Tama sil	6.0	3.04	0.272	23.8
6	Primghar sicl	7.0	3.41	0.313	33.2
7	Clyde sil	5.5	4.30	0.402	29.9
8	Glencoe sic	8.0	9.60	0.910	58.5

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[2] Professor and Research Associate, respectively, Department of Agronomy, Iowa State Univ., Ames.

sieve. In the analyses reported in Table 1, total nitrogen was determined by a semimicro-Kjeldahl procedure (1), organic carbon by the method of Mebius (7), pH by a glass electrode (soil/water ratio, 1:2.5), and cation-exchange capacity by the ammonium acetate procedure described by Jackson (6).

METHODS FOR DETERMINATION OF EXCHANGEABLE AMMONIUM, NITRATE, AND NITRITE

Apparatus

Steam-distillation apparatus—The apparatus used has been described (3). It is designed so that flasks fitted with standard-taper (19/38) ground-glass joints can be used as distillation chambers. Before use, the apparatus should be steamed out for about 10 min to remove traces of ammonia, and the rate of steam generation should be adjusted so that about 7.5 ml of distillate are collected/min.

Distillation flasks—The flasks used are 100-ml Kjeldahl flasks fitted with a side-arm, standard-taper ground-glass joints, and glass hooks, the neck of the side-arm being fitted with a standard-taper polyethylene stopper (4). The flask dimensions should be such that, when the flasks are connected to the distillation apparatus, the distance between the tip of the steam inlet tube and the bottom of the flask is approximately 4 mm.

Reagents

Potassium chloride solution, approximately 2*M*—Dissolve 1,500 g of reagent-grade KCl in 8 liters of water and dilute the solution to 10 liters.

Magnesium oxide—Heat heavy magnesium oxide (U.S.P.) in an electric muffle furnace at 600 to 700C for 2 hr. Cool the product in a desiccator containing KOH pellets and store it in a tightly stoppered bottle.

Boric acid-indicator solution—Prepare as previously described (3).

Devarda alloy—Ball-mill reagent-grade alloy until the product will pass a 100-mesh screen and at least 75% of it will pass a 300-mesh screen (4).

Sulfamic acid—Dissolve 2 g of purified sulfamic acid (4) in 100 ml of water. Store the solution in a refrigerator.

Sulfuric acid—0.005*N* standard.

Standard (ammonium + nitrate)-N solution—Dissolve 0.236 g of ammonium sulfate and 0.361 g of potassium nitrate in water, dilute the solution to 1,000 ml in a volumetric flask, and mix the solution thoroughly. If pure, dry reagents are used, this solution contains 50 μg of ammonium-N and 50 μg of nitrate-N/ml. Store the solution in a refrigerator.

Standard (ammonium + nitrate + nitrite)-N solution—Dissolve 0.236 g of ammonium sulfate, 0.123 g of sodium nitrite, and 0.361 g of potassium nitrate in water, dilute the solution to 1,000 ml in a volumetric flask, and mix the solution thoroughly. If pure, dry reagents are used, this solution contains 50μg of ammonium-N, 50μg of nitrate-N, and 25 μg of nitrite-N/ml. Store the solution in a refrigerator.

Procedure

Preparation of Soil Extract

Place 10 g of soil in a 250-ml, wide-mouth bottle and add 100 ml of 2*M* KCl. Stopper the bottle and shake it on a mechanical shaker for 1 hr. Then allow the soil-KCl suspension to settle until the supernatant liquid is clear (usually about 30 min) and perform the analyses described on aliquots of this liquid. If the KCl extract cannot be analyzed soon after its preparation (within 24 hr), filter the soil-KCl suspension (Whatman no. 42 filter paper) and store the filtrate in a refrigerator until analyses can be performed.

Analysis of Extract When Nitrite is Present

Ammonium-N—Add 5 ml of boric acid-indicator solution to a 50-ml Erlenmeyer flask marked to indicate a volume of 30 ml and place the flask under the condenser of the steam-distillation apparatus so that the end of the condenser is about 4 cm above the surface of the boric acid. Pipette an aliquot (usually 20 ml) of the soil extract into a distillation flask and add 0.2 g of MgO through a dry powder funnel with a stem reaching into the bulb of the flask. Attach the flask to the distillation apparatus by spiral steel springs with loop ends and commence distillation by closing the stopcock on the steam-bypass tube of the distillation apparatus. When the distillate reaches the 30-ml mark on the receiver flask, stop the distillation by opening the stopcock on the steam-bypass tube, rinse the end of the condenser, and determine ammonium-N in the distillate by titration with 0.005*N* H_2SO_4 from a 5-ml microburette graduated at 0.01-ml intervals (1 ml of 0.005*N* H_2SO_4 = 70 μg of NH_4-N). The color change at the end-point is from green to a permanent, faint pink.

(Nitrate + nitrite)-N—After removal of ammonium-N from the sample as described in the previous section, remove the stopper from the side-arm of the flask, add 0.2 g of Devarda alloy through a dry powder funnel with a stem reaching into the flask about 1 cm below the base of the ground joint on the side-arm, and immediately replace the stopper in the neck of the side-arm. Then determine the ammonia-N liberated by steam distillation as described in the previous section.

(Ammonium + nitrate + nitrite-N—Follow the procedure described for determination of ammonium-N, but add 0.2 g of Devarda alloy to the distillation flask immediately after addition of MgO and before connection of the flask to the distillation apparatus.

(Ammonium + nitrate)-N—Follow the procedure described for determination of (ammonium + nitrate + nitrite)-N, but before addition of MgO and Devarda alloy, treat the sample in the distillation flask with 1 ml of sulfamic acid solution and swirl the flask for a few seconds to destroy nitrite before connecting it to the distillation apparatus.

Nitrate-N—Follow the procedure described for determination of (nitrate + nitrite)-N, but perform the analysis on a sample that has been treated with sulfamic acid to destroy nitrite as in the procedure for determination of (ammonium + nitrate)-N.

Analysis of Extract When Nitrite is Absent

Ammonium-N—Follow the procedure described for determination of ammonium-N in the presence of nitrite.

Nitrate-N—Follow the procedure described for determination of (nitrate + nitrite)-N in the presence of nitrite.

(Ammonium + nitrate)-N—Follow the procedure described for determination of (ammonium + nitrate + nitrite)-N in the presence of nitrite.

Control and Check Analyses

Controls should be performed in each series of analyses to allow for ammonium-N derived from the reagents used, and the steam-distillation procedures should be checked at intervals by analyzing 5-ml aliquots of the standard (ammonium + nitrate)-N and (ammonium + nitrate + nitrite)-N solutions. Both solutions are stable for several months if stored in a refrigerator.

Distillation for N^{15} Analysis

If the ammonium liberated by distillation in the various methods of analysis described is to be analyzed for N^{15}, modify the distillation technique as described by Bremner and Edwards (3).

RESULTS AND DISCUSSION

Unless otherwise specified, all analytical data reported represent the means of results of replicate analyses which were in close agreement.

Table 2—Ammonium-N extracted by shaking incubated soils with KCl solution (10 ml/g of soil) for 1 hr

Soil no.	Molarity of KCl solution					
	0.125	0.25	0.50	1.0*	2.0	4.0†
	Ammonium-N extracted, ppm of soil					
1	21	23	24	26 (27)	27	27 (27)
2	68	76	80	81 (82)	82	82 (82)
3	85	94	95	96 (98)	98	98 (98)
4	39	43	46	49 (52)	52	52 (52)
8	230	237	242	246 (252)	252	252 (252)

* Figures in parentheses are values obtained using 20 ml of 1.0M KCl/g of soil.
† Figures in parentheses are values obtained using 5 ml of 4.0M KCl/g of soil.

Table 3—Ammonium-N extracted by shaking incubated soils with different volumes of 2M KCl for 1 hr

Soil no.	Milliliters of 2M KCl/g of soil			
	2	5	10	20
	Ammonium-N extracted, ppm of soil			
1	24	26	27	27
2	79	82	82	82
3	94	95	98	98
4	44	49	52	52
8	238	244	252	252

Extraction Procedure

Development—Since preliminary work using soils 2, 5, 6, and 7 showed that nitrate and nitrite added to these soils were readily extracted by a short (5- to 10-min) period of shaking with water or dilute KCl solution, the problem of developing an extraction procedure for determination of exchangeable *ammonium, nitrate, and nitrite* was basically that of finding a satisfactory KCl treatment for extraction of exchangeable ammonium. The extraction procedure recommended developed from studies using different volumes and concentrations of KCl solution and different shaking times. These studies showed that, when an equilibrium extraction technique is used, the amount of ammonium extracted from soils by KCl solution at room temperature is essentially maximal if 2*M* KCl is used and the soil sample is shaken with this reagent for 1 hr using 10 ml of 2*M* KCl/g of soil. This is illustrated by the data in Tables 2, 3, and 4, which show the effects of varying the concentration of the KCl solution used for extraction, the volume of 2*M* KCl/g of soil, and the time of shaking with 2*M* KCl on the amount of ammonium extracted by KCl solution from various soils. To ensure accurate evaluation of these effects, the soils studied were incubated under waterlogged conditions at 30C for 4 weeks and air-dried before use so that they would contain an appreciable amount of exchangeable ammonium. It can be seen that the amount of ammonium extracted by the 2*M* KCl technique recommended (10 ml of 2*M* KCl/g of soil) was identical to the amount extracted by 1*M* KCl using 20 ml/g of soil or by 4*M* KCl using 5 ml/g of soil (Table 2), and that it was not increased if either the volume of 2*M* KCl or the time of shaking recommended was increased (Tables 3 and 4). In other words, these studies showed that the amount of ammonium extracted by shaking soil with KCl solution at room temperature for 1 hr is the same whether 1*M*, 2*M*, or 4*M* KCl is used, provided that the amount of KCl solution employed contains the equivalent of 20 meq of K/g of soil; and that it is not increased if the meq of K/g of soil exceeds this value or if the time of shaking is increased. The 2*M* KCl extraction procedure was adopted because use of 2*M* KCl instead of 1*M* KCl reduces the size of the aliquot of extract required for satisfactory analysis by the steam distillation methods described, and analyses of several soils showed that the results obtained using 2*M* KCl for extraction had slightly higher precision than those obtained using 3*M* KCl or 4*M* KCl.

Table 4—Ammonium-N extracted by shaking incubated soils with 2M KCl (10 ml/g of soil) for different times

Soil no.	Shaking time, min					
	15	30	45	60	120	480
	Ammonium-N extracted, ppm of soil					
1	26	27	27	27	27	27
2	81	82	82	82	82	82
3	96	97	97	98	98	98
4	50	51	52	52	51	52
8	237	249	251	252	253	252

Table 5—Analyses of filtered and unfiltered soil extracts by steam-distillation methods described

Soil no.	Extract*	Method†				
		1	2	3	4	5
		μg of N, 20 ml of extract				
1	F	9	4	13	4	13
	U	9	4	13	4	13
2	F	8	4	12	4	12
	U	8	4	12	4	12
3	F	26	18	44	18	44
	U	26	18	44	18	44
	F‡	26	217	243	418	443
	U‡	26	218	244	417	444
4	F	8	4	12	4	12
	U	8	4	12	4	12
	F‡	8	204	212	405	412
	U‡	8	205	211	404	412
5	F	70	6	76	6	77
	U	70	6	76	6	76
8	F	16	6	22	6	22
	U	17	6	22	6	22

* Extracts were prepared by shaking soil samples with 2M KCl (10 ml/g of soil) for 1 hr. F, filtered extract; U, unfiltered extract. Analyses were performed on 20-ml aliquots of extracts.
† 1, ammonium-N; 2, nitrate-N; 3, (ammonium + nitrate)-N; 4, (nitrate + nitrite)-N; 5, (ammonium + nitrate + nitrite)-N.
‡ Aliquot of extract (20 ml) was treated with 5 ml of solution containing 200μg of nitrate-N (as KNO_3) and 200 μg of nitrite-N (as $NaNO_2$) immediately before analysis.

Filtration—Soil extracts prepared by the procedure recommended need not be filtered for analysis by the steam-distillation methods described (Table 5). Moreover, the aliquot of unfiltered extract taken for analysis need not be free of suspended soil material because the distillation methods of analysis described are not affected by this material. However, pipettes with wide tips should be used to sample supernatant liquids that are not clear, because pipettes with fine tips are liable to become clogged by suspended material. Filtration is recommended if the extract must be stored for some time before analysis because filtered extracts can be stored safely for several weeks if kept in a refrigerator (Table 7).

Recovery tests—Table 6 shows results of studies to determine the recoveries by the methods described of ammonium,

Table 6—Recoveries of ammonium-N, nitrate-N, and nitrite-N by methods described

Soil No.	Recovery of N added to soil, %*						Recovery of N added to soil extracts, %		
	NH_4-N		NO_3-N		NO_2-N		NH_4-N	NO_3-N	NO_2-N
	A	B	A	B	A	B			
1	99.5	91.0	100.6	100.6	99.8	94.9	100.1	99.6	100.0
2	100.5	84.5	99.6	99.6	99.8	99.1	100.0	99.9	99.8
3	99.5	95.4	100.5	100.5	99.6	95.5	100.2	99.8	100.2
4	99.8	79.8	99.6	100.4	99.7	96.6	99.8	100.1	100.1
5	99.6	97.4	99.1	99.4	100.6	98.4	100.1	99.6	100.2
6	99.8	96.6	100.0	99.8	99.5	98.5	99.8	99.9	99.9
7	100.0	92.7	100.1	99.6	99.5	93.0	100.0	99.9	99.8
8	99.8	87.2	99.9	99.6	99.9	97.4	100.0	99.9	99.8
Avg.	99.8	90.6	99.9	100.0	99.8	96.7	100.0	99.8	100.0

* A, standard (ammonium + nitrate + nitrite)-N solution was added to soil immediately after addition of KCl solution; B, standard (ammonium + nitrate + nitrite)-N solution was added to soil immediately before addition of KCl solution.

nitrate, and nitrite added to various soils. Recoveries were determined by analyzing extracts obtained by shaking 10 g of soil with 100 ml of 2*M* KCl and 10 ml of standard (ammonium + nitrate + nitrite)-N solution for 1 hr, the latter solution being added immediately before or after the addition of 2*M* KCl, and by subtracting the ammonium-N, nitrate-N, and nitrite-N values obtained in analyses of extracts obtained by shaking 10 g of soil with 100 ml of 2*M* KCl and 10 ml of water for 1 hr. It can be seen that the methods described gave quantitative recoveries of ammonium-N, nitrate-N, and nitrite-N added to soils after addition of KCl, and that, when these forms of nitrogen were added before addition of KCl, their recoveries averaged 90.6, 100.0, and 96.7%, respectively. Failure to achieve quantitative recovery of ammonium added before addition of KCl is readily explicable on the grounds that the soils used had the capacity to fix ammonium. Work in progress indicates that the failure to obtain completely quantitative recovery of nitrite-N added before addition of KCl is due to chemical decomposition of nitrite by soil constituents.

Table 6 also shows data obtained in studies to determine the recoveries by the steam-distillation procedures described of ammonium, nitrate, and nitrite added to 2*M* KCl extracts of soils. The extracts used were prepared by shaking 10 g samples of soils with 100 ml of 2*M* KCl for 1 hr and filtering the resulting suspensions, and the recoveries reported were calculated from analyses of 20-ml aliquots of these extracts before and after addition of 5 ml of standard (ammonium + nitrate + nitrite)-N solution. Exchangeable ammonium-N, nitrate-N, and (nitrate + nitrite)-N were determined by the procedures recommended for determination of these forms of nitrogen in the presence of nitrite, and nitrite-N was calculated by difference. It can be seen that the distillation procedures described gave quantitative recoveries of ammonium-N, nitrate-N, and nitrite-N added to KCl extracts of all soils studied. Similar studies showed that these distillation procedures also gave quantitative recoveries of ammonium-N, nitrate-N, and nitrite-N added to water, NaCl, and K_2SO_4 extracts. The standard (ammonium + nitrate + nitrite)-N solution used in the recovery tests reported contained 200 μg/ml of ammonium-N [as $(NH_4)_2SO_4$], nitrate-N (as KNO_3), and nitrite-N (as $NaNO_2$).

Changes during extraction—To be satisfactory, a method used to prepare soil extracts for determination of exchangeable ammonium, nitrate, or nitrite should neither cause nor permit biological or nonbiological reactions which lead to an increase or decrease in the amounts of these forms of nitrogen. The recovery tests reported in Table 6 indicate that the extraction procedure recommended meets this requirement, and other tests performed support this conclusion. We found, for example, that the results of soil analyses by the methods described are not affected if the 2*M* KCl used for extraction is treated with toluene to inhibit microbial transformations of inorganic forms of nitrogen during extraction. We also tested the possibility that use of a neutral extractant such as 2*M* KCl might lead to chemical decomposition of nitrite during extraction of acidic soils or to volatilization of ammonium during extraction of calcareous soils, but were unable to detect such reactions in recovery studies using soil 3 (pH 4.8) and samples of soils 1, 2, and 8 treated with $CaCO_3$ (10% by weight). Nevertheless, we recommend that the extraction procedure recommended be checked by recovery tests if it is to be applied to highly acidic or calcareous soils and quantitative analyses for nitrite and exchangeable ammonium are desired. The literature on determination of nitrite in soils shows that the instability of nitrite in acidic media has not been fully appreciated and that several workers have used acidic reagents for extraction of nitrite (2).

The possibility that analyses of soils for inorganic forms of nitrogen may be vitiated by enzymatic reactions during preparation of extracts for these analyses deserves attention because it is now well established that soils contain a variety of enzymes capable of transforming nitrogenous compounds. Dashevskiy (5) obtained some evidence that the determination of exchangeable ammonium in soils may be complicated by enzymatic hydrolysis of amides during preparation of extracts for this determination. We have been unable to confirm his findings using unamended soils, but have been able to detect enzymatic activity during extraction of soils treated with urea. We found, for example, that the values obtained in analyses of soils 5 and 6 for exchangeable ammonium-N by the extraction-distillation procedure described increased significantly (4 to 8 ppm) when these soils were treated with urea (200 ppm of N) immediately before analysis.

Storage of extracts—Studies using field-moist and air-dried soils showed that the stability of extracts obtained by the procedure recommended is markedly increased if the extracts are filtered before storage, but that unfiltered extracts can be stored safely for up to 24 hr at 25 C and for up to 48 hr at 5C. Filtered extracts can be stored for at least 45 days at 25C

Table 7—Effect of storing filtered and unfiltered soil extracts on the values obtained in their analysis for ammonium, nitrate, and nitrite*

Storage		Filtered extract			Unfiltered extract		
Temperature	Time	NH_4-N	NO_3-N	NO_2-N	NH_4-N	NO_3-N	NO_2-N
°C	days	μg of N/20 ml of extract					
25	0	108	103	50	108	103	50
	1	107	104	51	108	102	51
	2	108	103	50	107	103	41
	4	109	102	50	111	104	36
	7	107	91	41	113	94	29
	14	108	80	32	130	84	24
	21	108	70	26	161	73	19
5	0	107	101	51	107	103	51
	1	108	103	50	107	103	51
	2	108	103	51	108	103	51
	4	109	104	50	110	104	50
	7	108	102	50	113	102	42
	14	107	104	51	129	87	37
	21	108	103	50	140	69	32
	120	107	103	51	—	—	—

* Extracts were prepared by shaking 60 g samples of soil 4 with 600 ml of 2M KCl solution containing 3 mg of ammonium-N [as $(NH_4)_2SO_4$], 3 mg of nitrate-N (as KNO_3), and 1.5 mg of nitrite-N (as $NaNO_2$) for 1 hr.

and for up to 4 months if kept in a refrigerator at 5C. Table 7 shows data obtained in a study of the effect of storing 2M KCl extracts of Webster soil.

Steam-Distillation Procedures

Specificity and interference tests—Tests using 69 organic nitrogen compounds, including amino acids, purine and pyrimidine derivatives, and alkali-labile substances such as amides and hexosamines, showed that the steam-distillation methods of analysis described had high specificity and that only two compounds, namely glucosamine and galactosamine, yielded detectable amounts of ammonium under the conditions of these methods (4). If a soil extract contained a significant amount of glucosamine or galactosamine, interference by these hexosamines in analyses for ammonium, nitrate, and nitrite could be eliminated by use of the methods for ammonium-N, (ammonium + nitrate)-N, and (ammonium + nitrate + nitrite)-N, and reduction of the time of distillation in the method for ammonium-N to 2 min (4). However, hexosamines are so labile in alkaline media that their presence in an extract analyzed for ammonium by steam distillation with MgO can readily be detected by studying the effects of varying the amount of MgO and the time of distillation (4), and such studies showed that hexosamines are either completely absent from KCl extracts of soils or are present in such low concentration that they do not release measurable amounts of ammonium under the conditions of the MgO distillation method recommended for ammonium analysis. The finding that KCl extracts of soils do not contain organic nitrogen compounds which yield ammonium in alkaline media as rapidly as hexosamines is illustrated by Table 8 which shows the amounts of ammonium liberated by steam distillation of 2M KCl extracts of soils with various alkaline reagents. It can be seen that the amount of ammonium liberated by steam distillation with MgO was not affected if the amount of MgO used was increased from 0.1 to 1.0 g or if the time of distillation was increased from 2 to 6 min; and that the amount liberated by distillation with a mildly alkaline

Table 8—Ammonium-N liberated by steam distillation of soil extracts with various reagents

Reagent	Time of distillation, min†	Extract*		
		Soil 3	Soil 4	Soil 8
		Ammonium-N liberated, μg/20 ml of extract		
MgO (0.1 g)	2	26	8	16
	R	26	8	16
	6	26	8	16
MgO (0.2 g)	2	26	8	16
	R	26	8	16
	6	26	8	16
MgO (1.0g)	2	26	8	16
	R	26	8	16
	6	26	8	16
MgO (0.2g) + Devarda alloy (0.2g)	2	44	12	22
	R	44	12	22
	6	44	12	22
Potassium phosphate buffer, 1M, pH 8.0 (10 ml)	2	26	8	16
	R	26	8	16
	6	26	8	16
CaO (0.2g)	2	26	8	16
	R	26	8	16
	6	26	8	16
NaOH (0.2g)	2	32	12	20
	R	34	14	26
	6	35	15	28
NaOH (1.0g)	2	36	18	38
	R	44	24	50
	6	46	26	52

* Extracts were prepared by shaking soil samples with 2M KCl (10 ml/g of soil) for 1 hr. Analyses were performed on 20-ml aliquots of filtered extracts.
† R, time used in methods described (ca. 3.3 min); rate of distillation, ca. 7.5 ml/min.

potassium phosphate buffer (pH 8.0) or with CaO was identical to the amount released by MgO distillation. The data in Table 8 also show that the results obtained by the MgO-Devarda alloy distillation method of determining (ammonium + nitrate + nitrite)-N were not affected if the period of distillation was increased to 6 min. Other studies showed that the results obtained with soil extracts by the methods for determination of nitrate-N, (ammonium + nitrate)-N, and (nitrate + nitrite)-N were similarly unaffected if the time of distillation was increased to 6 min. The results using NaOH for distillation merely confirm previous evidence that soil extracts contain organic nitrogen compounds which decompose with formation of ammonium when distilled with strong alkali.

Studies of the effects of various cations and anions on the steam-distillation methods of analysis described have already been reported (4), and it suffices here to state that extensive tests indicate that these methods are not subject to interference by inorganic or organic substances present in soil extracts. This conclusion is supported by the findings that these methods give quantitative recovery of ammonium, nitrate, and nitrite added to soil extracts (Table 6) and that the results obtained with soil extracts by these methods are not affected if the sample of extract analyzed contains suspended soil material (Table 5). Work reported in the next paper in this series has shown that these steam distillation methods of analysis have such high specificity and are so unaffected by soil constituents that it is possible to apply them directly to most types of soils (i.e., eliminate the extraction step in the procedures described). It should be noted, however, that the reduction of nitrate and nitrite by

Table 9—Precision of methods

Amount of soil analyzed, g*	Method†	μg N/g of soil‡		
		Range	Average	Standard deviation
10.0	1	11–12	12	1.0
	2	53–55	54	1.4
	3	65–67	66	1.5
	4	53–55	54	1.4
	5	64–67	66	1.5
5.0	1	11–12	12	1.1
	2	54–55	54	1.4
	3	65–67	66	1.1
	4	53–54	54	1.3
	5	65–67	66	1.5
2.0	1	11–12	12	1.2
	2	53–55	54	1.5
	3	64–67	66	1.6
	4	53–54	54	1.3
	5	65–66	66	1.2

* Extracts were prepared by shaking soil samples with 2M KCl (10 ml/g of soil) for 1 hr. Analyses were performed on 20-ml aliquots of filtered extracts.
† 1, exchangeable ammonium-N; 2, nitrate-N; 3, (exchangeable ammonium + nitrate)-N; 4, (nitrate + nitrite)-N; 5, (exchangeable ammonium + nitrate + nitrate)-N.
‡ Results of 6 analyses of soil 6 by each method.

Devarda alloy and MgO is affected by the presence of an appreciable concentration of dissolved phosphate or silicate (4), and that the methods of analysis involving use of Devarda alloy cannot therefore be applied to soil extracts obtained by treating soils with phosphate or silicate solutions.

Accuracy and precision—The steam-distillation procedures described give quantitative results when applied to 20-ml aliquots of solutions containing up to 100 μg/ml of ammonium-N, nitrate-N, and nitrite-N (4), and give quantitative recovery of ammonium-N, nitrate-N, and nitrite-N added to soil extracts (Table 6).

The high precision of the extraction-distillation methods of analysis is illustrated by Table 9, which shows results obtained by these methods in replicate analyses of air-dried Primghar soil (< 2 mm). The data in Table 9 also show that the results by these methods using 2 or 5 g of soil were identical to, and as precise as, those obtained using 10 g of soil. Tests using soils 1, 3, 4, and 6 showed that the results obtained by the extraction-distillation methods with field-moist, < 2 mm samples of these soils were as precise as those obtained with air-dried samples.

Apparatus and reagents—Description of the steam-distillation methods has been simplified by specification that distillation flasks fitted with a side-arm be used. This side-arm is not required when the method of analysis does not involve addition of Devarda alloy after removal of ammonium by steam distillation with MgO.

The MgO used for distillation of ammonium is ignited to remove carbonate and stored in an air-tight container to protect it from atmospheric CO_2 because use of MgO containing carbonate can lead to liberation of CO_2 which interferes with determination of ammonium by titration methods.

The Devarda alloy used for reduction of nitrate and nitrite must be ball-milled to obtain a very finely divided product because the reactivity of this alloy increases with decrease in its particle size, and finely divided alloy must be used to obtain quantitative reduction of nitrate and nitrite within the period of distillation recommended (4).

Calibrated glass spoons (9) can be employed for addition of MgO and Devarda alloy because it is not necessary to use accurately weighed amounts of these reagents.

Reagent-grade sulfamic acid sometimes contains an appreciable quantity of ammonium, and it usually requires purification for use in the methods described. Sulfamic acid is hydrolyzed in aqueous solution with formation of ammonium bisulfate ($NH_2SO_3H + H_2O = NH_4HSO_4$), but this reaction is very slow at 5C, and only trace amounts of ammonium have been detected in sulfamic acid solutions prepared as recommended and subsequently stored in a refrigerator for 6 months.

Additional information concerning the apparatus and reagents used can be found in previous papers (3, 4).

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