

## Distillation and Microdiffusion Modifications for total $\text{NH}_{4f}$ Quantification and $^{15}\text{N}$ Recovery

Gaius D. Eudoxie and Gregory A. Gouveia

Department of Food Production, Faculty of Sciences and Agriculture,  
The University of the West Indies, St. Augustine Campus, Trinidad

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**Abstract: Problem Statement:** Applying  $^{15}\text{N}$  techniques to accurately determine the fate of fixed ammonium ( $\text{NH}_{4f}$ ) in the strongly and weakly held pools require modifications to existing methodologies. Modifications are necessary for measurement of total  $\text{NH}_{4f}$  in soils by direct digestion with 5 M HF: 1 M HCl, excluding alkali pretreatment, followed by distillation and quantification of  $\text{NH}_4$ . Quantification by distillation was used as a precursor to optimize microdiffusion protocols for continuous flow-isotope ratio mass spectrometry (CF-IRMS). This paper reports on the modifications applied to these procedures since the direct 5 M HF: 1 M HCl digestion of soil samples may also dissolve some organic N fractions. **Approach:** Distillation followed by  $^{15}\text{N}$  microdiffusion trials were conducted on soil digests amended with rice husks, manure, compost or glycine, using different molarities (2, 5, and 10 M) and volumes (5, 10, 15, 25, 32.5, and 40 mL) of KOH. **Results:** The distillation study identified 32.5 mL of 2 M KOH to be the optimum volume and molarity of KOH that must be combined with 10-mL aliquots of direct 5 M HF: 1 M HCl digests of each of seven soils to ensure that only  $\text{NH}_4$  in the digest is recovered and none of the organic N is hydrolyzed during the process. Results also showed that a minimum incubation time of 192 h was needed to trap approximately 100  $\mu\text{g}$   $^{15}\text{N}$  on the disks for subsequent accurate analysis by CF-IRMS, with minimal recovery of organic N. **Conclusions/Recommendations:** These findings support the use of a direct-digestion/distillation method to quantify total  $\text{NH}_{4f}$  and thereby provide opportunity to distinguish between strongly and weakly held  $\text{NH}_{4f}$  in soils.

**Key words:** Weakly held  $\text{NH}_{4f}$ , strongly held  $\text{NH}_{4f}$

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### INTRODUCTION

Ammonium fixed in the lattices of certain clay minerals has been investigated extensively since the observation<sup>[1]</sup> that HF pre-treated soil samples yielded higher total N values than those treated solely with  $\text{H}_2\text{SO}_4$ . Research on the fixation and release dynamics of  $\text{NH}_4$  and the factors influencing its behavior<sup>[2,3]</sup> suggests that once fixed, this  $\text{NH}_4$  remains essentially unavailable and no longer participates in the N cycle.

Paramasivam and Breitenbeck<sup>[4]</sup> alluded to the inability of previous studies to adequately quantify total fixed  $\text{NH}_4$  ( $\text{NH}_{4f}$ ). They demonstrated that the widely used method<sup>[5]</sup>, which involved pre-treatment of soil samples with alkaline KOBr before the addition of the HF-HCl acid mixture, did not account for  $\text{NH}_4$  that was fixed weakly near the edges of clay minerals. This component of the total  $\text{NH}_{4f}$  was removed with the KOBr washing along with organic N and extractable ammonium ( $\text{NH}_{4e}$ ) and could only be determined by calculating the difference in the amounts obtained quantitatively from the two methods. A proposed sub-classification<sup>[6]</sup> of total  $\text{NH}_{4f}$  into strongly and weakly held fractions was

suggested and predicted expected differences in fraction behavior, which would have some influence on N availability and management in soils. Significant quantities of recently  $\text{NH}_{4f}$  could be weakly held<sup>[4]</sup> with a possible shorter existence, which may have tremendous implications for N management particularly on high-activity clayey soils.

Since strongly held  $\text{NH}_{4f}$  in soil-acid digests<sup>[5]</sup> were not concentrated enough to allow direct injection into automated  $^{15}\text{N}$  analyzers, a microdiffusion protocol<sup>[6]</sup> was developed imitating in many aspects the original specimen container<sup>[7]</sup>. Though somewhat small for the required volume for microdiffusion of the extractable N fraction, the specimen container is suitable for soil-acid digest since soils usually contain higher levels of  $\text{NH}_{4f}$  and the concentration of N in the digest would be greater. Additionally, HF would decompose glass jars so polymer containers are therefore necessary.

Laboratories with automated  $^{15}\text{N}$  analyzers can now conduct  $^{15}\text{N}$  analysis on the strongly held  $\text{NH}_{4f}$  fraction in soils using that simple method.  $^{15}\text{N}$  assessment of the total  $\text{NH}_{4f}$  can be calculated from  $^{15}\text{N}$  analysis of  $\text{NH}_{4e}$ <sup>[8,9]</sup> and strongly held  $\text{NH}_{4f}$ , but would underestimate the true

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**Corresponding Author:** Gaius D. Eudoxie, Department of Food Production, Faculty of Sciences and Agriculture, The University of the West Indies, St. Augustine Campus, Trinidad, Tel # (868) 662 2002 ext 3515, Fax # (868) 645-0479

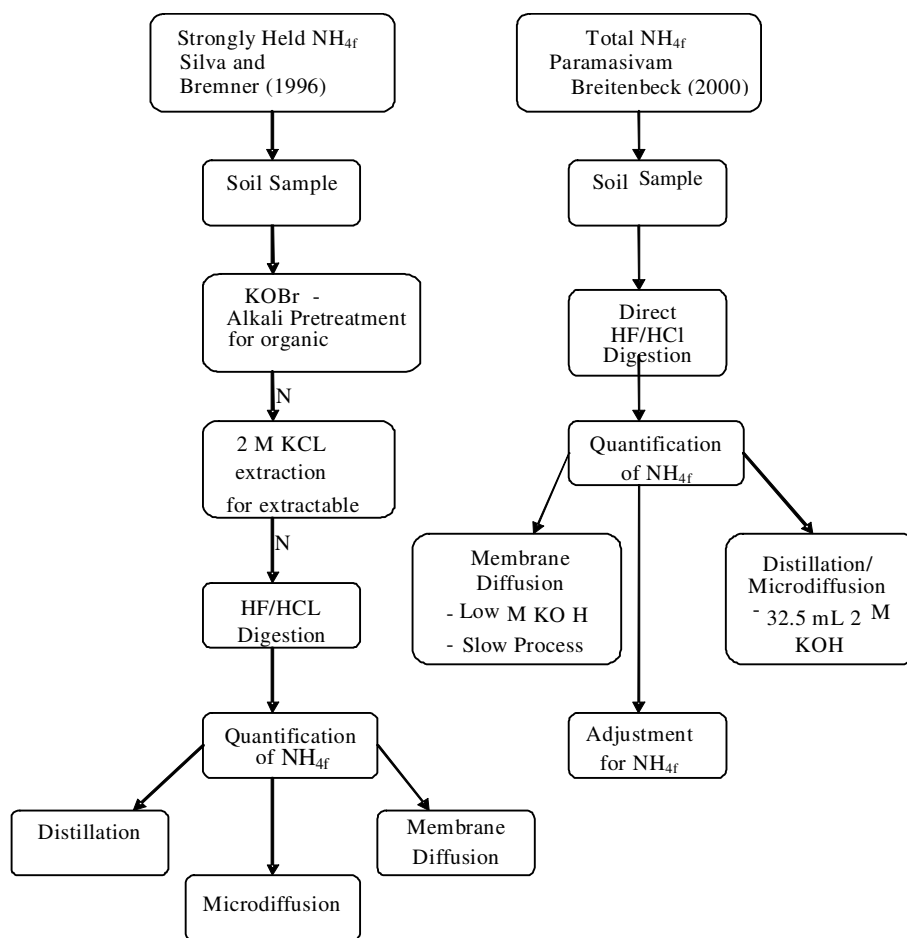


Fig. 1: Flowcharts for the determination of strongly held and total  $\text{NH}_4\text{f}$ . Authors' modifications highlighted in bold. The difference between the two procedure yields weakly held  $\text{NH}_4\text{f}$

quantity it  $\text{NH}_4$  was held in weakly fixed positions. Hence in laboratories with automated  $^{15}\text{N}$  analyzers, a microdiffusion technique for pre-concentrating ammonium from the total  $\text{NH}_4\text{f}$  digests had to be developed and validated (Fig. 1). This digest differs from that of strongly held  $\text{NH}_4\text{f}$  since organic N fractions are also dissolved and quantitative methods must minimize recovery of organic N. A  $\text{NH}_3$ -membrane diffusion technique<sup>[4]</sup> can be used to quantify this total  $\text{NH}_4\text{f}$ , however, for automated  $^{15}\text{N}$  analysis,  $\text{NH}_4$  in the digest would first have to be pre-concentrated onto glass-fiber disks using microdiffusion. Given the need to avoid diffusion of any N fractions in the digest other than the fixed and extractable  $\text{NH}_4$ , microdiffusion protocols must ensure that only these fractions are diffused to increase accuracy in the  $^{15}\text{N}$  analysis.

This study show data validating a modified microdiffusion procedure (Fig. 1) for pre-concentrating  $\text{NH}_4$  in the HF: HCl digest of a wide range of soils for  $^{15}\text{N}$  analysis by CFIR-MS using an automated N and C analyzer (ANCA, Model NA 1500, Carlo Erba Strumentazione) interfaced with a mass spectrometer (MS, Model Tracermass-Stable Isotope Analyzer, Europa Scientific)<sup>[10]</sup>. Studies were conducted to optimize the volume and molarity of KOH necessary for the distillation of the  $\text{NH}_4$  from the digests without recovery of labile organic N. These alkali specifications were then tested in the microdiffusion of  $\text{NH}_4$  from the digests with additions of various types and concentrations of organic and inorganic spikes. Hereafter, any reference to total  $\text{NH}_4\text{f}$  analysis ( $^{15}\text{N}$  or otherwise) recognizes that the  $\text{NH}_4\text{c}$  is also present in the digest and must be accounted for in the calculation.

**MATERIALS AND METHODS**

**Soil Properties and Preparation of Direct Soil-Acid Digests:**

Seven soils were selected covering a range of physical, chemical and mineralogical properties and included one sandy soil, two loams, two clays, an acid sulphate soil and a peaty-loam soil (Table 1). The soils were collected in Trinidad in sites under fallow for greater than 10 y. Adequate quantities of the topsoil (0-15 cm) at each site were collected to facilitate characterization of the soil and the sequence of experiments that were conducted. Soil pH was determined on 1:2.5 (soil/H<sub>2</sub>O) suspensions with a glass electrode. Total N was determined by a modified Kjeldahl procedure<sup>[11]</sup>. Organic C content was measured by the modified Walkley-Black wet oxidation procedure<sup>[12]</sup>. Particle size distribution was determined by the hydrometer method<sup>[13]</sup>. Cation Exchange Capacity (CEC) was determined using the unbuffered salt extraction method<sup>[14]</sup>. Strongly held NH<sub>4f</sub> was measured using the KOH-KOBr pretreatment method<sup>[5]</sup> and total NH<sub>4f</sub> by a herein modified method (Fig. 1). Exchangeable NH<sub>4</sub> was determined by distillation of 2 M KCl soil extracts<sup>[8]</sup>.

The desired volumes of total NH<sub>4f</sub> digests for all soils were obtained by digestion of replicate samples<sup>[4]</sup>. In the procedure the soil (air dried and ground to < 100 µm) was treated directly with 5 M HF: 1 M HCl at a soil:solution

ratio of 1:20 in stoppered 100-mL centrifuge tubes. The suspension was incubated on a wrist hand shaker for 24 h, after which the supernatant was extracted through centrifugation and stored under ambient conditions for no longer than two days.

**Reagents and apparatus:** Reagents and apparatus used in these experiments have been described by these authors<sup>[6]</sup>.

**Study to optimize distillation procedure for total NH<sub>4f</sub> determination:**

Experiments were performed to determine the optimum molarity and volume of KOH necessary for quantification of total NH<sub>4f</sub>, which would minimize recovery of alkali-labile organic N during distillation. In these experiments, soil digests (10 mL) spiked with rice husks, manure, compost, glycine, or inorganic N, supplying different quantities and forms of N were treated with different molarities (2, 5 and 10 M) and volumes (5, 10, 15, 25, 32.5 and 40 mL) of KOH. A no-spike control was included to quantify the recovery of native NH<sub>4</sub> in the soil digests. The total number of digest/KOH/spike combinations was 120 for each soil. The amount of NH<sub>4</sub> liberated during distillation and trapped in the boric acid-indicator solution was determined by subsequent titration of the distillate with

Table 1: Physical and chemical properties of selected soils used in this investigation

Soil Series	Taxonomy	pH	Particle Size (g kg <sup>-1</sup> )				Organic C	Total N	C/N	CEC (cmol <sub>e</sub> kg <sup>-1</sup> )	NH <sub>4e</sub>	Strongly	Total
			Sand	Silt	Clay	NH <sub>4f</sub>						NH <sub>4f</sub>	
Piarco	Fine-sandy, kaolinitic, isohyperthermic, Aquoxic Tropudults	4.21	728	160	112	8.6	0.8	10.7	2.03	48.5	15.7	20.5	
Bejucal	Very fine, mixed, acid, isohyperthermic, Entic Pelluderts	4.23	78	110	812	14.1	2.4	5.9	15.9	72	405	405	
Princes Town	Very fine, montmorillonitic, nonacid, isohyperthermic Aquentic Chromuderts	7.07	212	170	618	21.0	2.2	9.5	26.9	36.6	204	204	
Montserrat	Fine, oxidic, isohyperthermic, Typic Tropudolls	6.84	288	130	582	21.7	2.9	7.5	22.5	29.8	143	144	
River Estate	Fine-loamy, micaceous, isohyperthermic, Fluventic Eutropepts isohyperthermic,	5.59	347	330	323	12.6	1.4	9.0	7.99	16.9	316	317	
Caroni	Very-fine, mixed, Typic Sulfaquents	3.06	347	270	383	33.6	2.4	14.0	22.8	156	407	408	
Chaguaramas <sup>a</sup>	Tropohemists	3.94	179	510	173	104	8.0	13.0	19.6	103	225	245	

<sup>a</sup> This soil is unknown and was only recently discovered in Chaguaramas, North Western Trinidad and does not fit the properties of any existing peaty soils and has not been fully classified

Table 2: Nitrogen recovered from 10 mL direct soil-HF/HCl digests of a range of soils amended with various N containing materials after distillation with 32.5 mL of 2 M KOH

Soil series	Materials added	Amount added g	Amount of N added (µg)	N recovered from distillation		
				Total	From amendment <sup>a</sup>	Recovery of added N (%)
Piarco	None	None	None	34.5 (1.1) <sup>b</sup>		
	Rice Husks	0.5	1644	56.8 (1.5)	0.0	0.00
	Manure	0.5	7103	82.0 (1.5)	1.6	0.02
	Compost	0.5	12953	276.0 (0.7)	5.5	0.04
	Glycine	0.5	89880	34.9 (0.7)	0.4	0.00
	NH <sub>4</sub> -N	-	250	280.0 (0.7)	246.0	98.30
Bejucal	None	None	None	238.0 (1.5)		
	Rice Husks	0.5	1644	265.0 (1.0)	2.2	0.14
	Manure	0.5	7103	289.0 (2.1)	5.1	0.07
	Compost	0.5	12953	481.0 (1.5)	7.0	0.06
	Glycine	0.5	89880	240.0 (1.0)	2.0	0.00
	NH <sub>4</sub> -N	-	250	483.0 (0.6)	245.0	98.00
Princess Town	None	None	None	119.0 (0.0)		
	Rice Husks	0.5	1644	141.0 (0.0)	0.0	0.00
	Manure	0.5	7103	168.0 (1.3)	2.9	0.04
	Compost	0.5	12953	361.0 (2.0)	6.0	0.05
	Glycine	0.5	89880	120.0 (0.5)	1.0	0.00
	NH <sub>4</sub> -N	-	250	366.0 (0.7)	247.0	99.00
Montserrat	None	None	None	84.3 (0.7)		
	Rice Husks	0.5	1644	109.0 (0.6)	0.0	0.00
	Manure	0.5	7103	134.0 (0.0)	3.8	0.05
	Compost	0.5	12953	324.0 (1.0)	3.7	0.03
	Glycine	0.5	89880	87.0(0.7)	2.7	0.00
	NH <sub>4</sub> -N	-	250	325.0 (0.6)	241.0	96.40
River Estate	None	None	None	165.0 (1.6)		
	Rice Husks	0.5	1644	187.0 (1.9)	0.0	0.00
	Manure	0.5	7103	216.0 (1.2)	5.1	0.07
	Compost	0.5	12953	403.0 (2.5)	2.0	0.02
	Glycine	0.5	89880	168.0 (0.8)	3.0	0.00
	NH <sub>4</sub> -N	-	250	409.0 (0.8)	244.0	97.60
Caroni	None	None	None	282.0 (0.0)		
	Rice Husks	0.5	1644	310.0 (1.5)	3.2	0.20
	Manure	0.5	7103	331.0 (2.1)	3.1	0.04
	Compost	0.5	12953	528.0 (0.5)	10.0	0.08
	Glycine	0.5	89880	282.0 (1.0)	0.0	0.00
	NH <sub>4</sub> -N	-	250	529.0 (0.5)	247.0	98.80
Chaguaramas	None	None	None	174.0 (0.5)		
	Rice Husks	0.5	1644	201.0 (1.7)	2.2	0.14
	Manure	0.5	7103	221.0 (1.8)	1.1	0.02
	Compost	0.5	12953	427.0 (1.5)	17.0	0.13
	Glycine	0.5	89880	175.0 (0.9)	1.0	0.00
	NH <sub>4</sub> -N	-	250	420.0 (0.8)	246.0	98.40

<sup>a</sup> NH<sub>4</sub> recovered from amendment is calculated as the difference between treated and control totals. In addition, for organically treated samples the quantities of 2 M KCl extractable NH<sub>4</sub> is removed. Quantities of extractable NH<sub>4</sub> is as follows: Rice husks - 24.8µg, Manure - 45.9µg, Compost -236 µg, Glycine -0 µg

<sup>b</sup> Standard deviation in parentheses

0.0025 M H<sub>2</sub>SO<sub>4</sub>. Total NH<sub>4f</sub> was determined as the difference between the NH<sub>4</sub> liberated after distillation of the direct soil-acid digest, excluding any organic-N and that recovered as NH<sub>4e</sub>. Hydrolyzed organic N was determined as the difference between NH<sub>4</sub> recovered from control units and that from units spiked with organic N.

**Study on microdiffusion of organically amended direct soil-acid digests:** In the previous experiments, an optimal molarity and volume of KOH that permitted quantitative recovery via distillation, of total NH<sub>4f</sub> without any significant alkali-labile organic N from the various soil digest was identified. These KOH specifications were validated in microdiffusion conditions in a series of

incubation experiments using similar organic N amendments.

Recovery of N via distillation differs from that of microdiffusion<sup>[9]</sup> particularly with respect to temperature, which influences incubation period. As a result much longer incubation periods are required for microdiffusion, which might result in greater recovery of labile organic N. These studies were a necessary precursor to <sup>15</sup>N microdiffusion studies, ensuring minimal organic N interference in recovery of total NH<sub>4f</sub>. Incubations were performed with 10-mL aliquots of direct soil-acid digests amended with 0.5 g of the same organic N compounds used in the distillation studies. Ammonium (200 µg N) was also applied as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the digests and a no-N control included to quantify native NH<sub>4</sub> in the soil digest. The specified volume (32.5 mL) of 2M KOH was placed in the units followed by the aliquot of soil-acid digests and quantity of N containing materials. Immediately 5 mL of 2 % H<sub>3</sub>BO<sub>3</sub> was added to a Petri-dish, which was secured on the screw-down mounting base. The containers were then fastened and incubated at room temperature for varying periods, at which time NH<sub>4</sub>-N was quantified by acid titration.

**Study on microdiffusion of organically amended direct soil-acid digests enriched with <sup>15</sup>N:** Quantification of total NH<sub>4f</sub> from soil-acid digests without recovery of labile organic N in microdiffusion conditions using the optimal molarity and volume of KOH was confirmed in the previous experiments. Similar incubations were performed on <sup>15</sup>N labeled soil-acid digests to determine the optimal periods of incubation for pre-concentration of total NH<sub>4f</sub> on acidified glass fiber disks.

In these studies 10-mL aliquots of the acid digests for each soil were spiked with 100 µg of <sup>15</sup>NH<sub>4</sub>-N as (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> having 10.6 % <sup>15</sup>N atom abundance. The specified volume of digest was dispensed into the units containing 32.5 mL of 2 M KOH. Two 7-mm diameter Whatman GF-D filter (Whatman International Ltd., Maidstone, UK) paper disks were each quickly acidified with 10 µL of 2.5 M KHSO<sub>4</sub> and the lid fastened tightly onto the container with a final gentle swirling to mix the contents. The units were incubated at room temperature (25°C) for similar diffusion periods, at which time assessment of the <sup>15</sup>N atom abundance and quantitative N recovery from the disks were determined.

The observed (O) percentage <sup>15</sup>N atom abundance values were compared to an expected (E) value, which

was calculated using the equation,  $E = (M_S A_S + M_F A_F) / (M_F + M_S)$ , where M is the mass of NH<sub>4</sub>-N in micrograms in the soil digest originating from soil (S) and from an addition of labeled fertilizer N (F) each with their own % <sup>15</sup>N atom abundance (A).

Using mass balance equations, the expected <sup>15</sup>N atom Abundance (A) of the total (TF) and weakly (WF) <sup>15</sup>NH<sub>4f</sub>-N fractions can be calculated as follows:

$$A_{TF} = (M_T A_T - M_E A_E) / (M_T - M_E)$$

$A_{WF} = (M_{TF} A_{TF} - M_{SF} A_{SF}) / (M_{TF} - M_{SF})$ , where T, E and SF represents total, exchangeable and strongly fixed NH<sub>4</sub>-N fractions respectively.

**Statistical Analyses:** All experiments were conducted with four replicates. Descriptive statistics were done to determine treatment means and accompanying Standard Deviations (SD) and Coefficients of Variation (CV).

## RESULTS

**Distillation of soil-acid digests spiked with organic and inorganic N:** Initial studies ascertained the quantity of NH<sub>4</sub> recovered after distillation from 10 mL of soil- acid digests for soil/ spike combinations. The absolute amounts of NH<sub>4</sub> recovered from the digests for each of the control (no amendments) soil treatments represent the combined total of NH<sub>4f</sub> and NH<sub>4e</sub> for any given soil. These were 34.5, 238, 119, 84.3, 165, 282 and 174 µg of NH<sub>4</sub>-N for the Piarco, Bejucal, Princes Town, Montserrat, River Estate, Caroni and Chaguaramas soil series, respectively (Table 2). The addition of 0.5 g of rice husks, manure, compost and glycine contributed a total amount of 1644, 7103, 12953 and 89880 µg N respectively to the digest. Of these totals 24.8, 45.9, 236 and 0 µg N would have been 2 M KCl NH<sub>4e</sub> which would have been recovered during distillation. The organic N recovered from the amendment during distillation was calculated as the difference between total NH<sub>4</sub> recovered (after accounting for NH<sub>4e</sub>) and the amount in the control soil (Table 2).

The optimum combination of volume and molarity of KOH was observed to be 32.5 mL of 2 M KOH (Table 3). Minimal amounts of organic N from the spikes were recovered after approximately 3 min of distillation with this KOH specification, whilst > 97% of the NH<sub>4</sub> spike was recovered.

Table 3: Recoveries of organic N from 10 mL of direct soil-HF/HCl digests of a range of soils spiked with four organic materials after distillation with KOH of different molarities and volumes

Soil Series	KOH molarity		Volume of KOH				
	M	5 mL	10 mL	15 mL	25 mL	32.5 mL	40 mL
Piarco	2	0 <sup>b</sup>	0	0	0	0	0-0.81
	5	0	0	0-0.37	0.02-2.47	ND	ND
	10	0	0.04-2.25	0.02-3.83	0.02-5.36	ND	ND
Bejucal	2	0	0	0	0	0-0.10	0-0.76
	5	0	0	0-0.1	0.01-2.13	ND	ND
	10	0	0.03-2.64	0.02-2.74	0.01-5.47	ND	ND
Princes Town	2	0	0	0	0	0	0-1.03
	5	0	0	0.01-0.28	0-2.22	ND	ND
	10	0	0-2.32	0.01-3.89	0.01-5.10	ND	ND
Montserrat	2	0	0	0	0	0-0.10	0-1.41
	5	0	0	0.01-0.35	0.01-2.46	ND	ND
	10	0	0.03-2.83	0.01-3.98	0.01-5.47	ND	ND
River Estate	2	0	0	0	0	0-0.10	0-1.27
	5	0	0	0-1.10	0.01-3.38	ND	ND
	10	0	0.01-3.01	0-4.46	0.01-6.24	ND	ND
Caroni	2	0	0	0	0	0-0.20	0.01-1.77
	5	0	0	0-0.94	0.01-2.62	ND	ND
	10	0	0.01-2.41	0.02-3.31	0.02-4.73	ND	ND
Chaguaramas	2	0	0	0	0	0-0.10	0.02-1.38
	5	0	0	0.03-1.97	0.04-2.91	ND	ND
	10	0	0.03-1.69	0.03-3.65	0.05-4.61	ND	ND

<sup>a</sup> Percentage organic N recovered is calculated as the difference between recoveries from spiked and control soil digests less 2 M KCl extractable N divided by the total organic N multiplied by 100

<sup>b</sup> The values represent the range of recoveries obtained across rice husks, compost, manure and glycine, with the lower value being that for glycine in all cases

ND: Not Determined - smaller volumes of similar molarity resulted in large organic N recoveries

Recovery of organic-N during distillation of soil/spike treatments, using >2 M KOH was notably greater, even where volumes were small (Table 3). Values ranging from 0-7.45% of the organic N from the amendments were recovered in distillations across the full range of combinations of KOH volume and molarity. The recovery of glycine N in the distillations never exceeded 0.06% even at the highest KOH volume/ molarity combination.

**Microdiffusion study of organically amended direct soil-acid digests:** Nitrogen recovered as total NH<sub>4</sub> from amended soil-acid digests increased steadily with time for all treatments (Table 4). The sampling period was selected to span 192 h<sup>[6]</sup>, allowing for the slower diffusion rate expected due to the larger volume<sup>[16]</sup> and lower molarity of alkali. Total NH<sub>4</sub> values after 192 h were similar to recovery via distillation. In most cases, diffusion resulted in only slightly higher quantities (not more than 108%). The quantity of NH<sub>4</sub>-N diffused was influenced by the initial NH<sub>4</sub> concentration in the digests with greater amounts being recovered for higher quantities at any diffusion period. Piarco and Montserrat soils showed zero recovery after 6 h for the native digests

where the presumed maximal N was less than 100 µg, whilst 45.0 and 41.9 µg were recovered for that same period for the aforementioned soils, where an additional 200 µg of N was added as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

Recoveries of NH<sub>4</sub>-N derived from organic amendments (not shown, but tabulated from Table 4) varied among the soils, but were less than 1%. Chaguaramas soil amended with rice husks resulted in the highest percent of organic N released. For all soils recovery of 200 µg NH<sub>4</sub>-N was >96% after 192 h.

**Microdiffusion of soil-acid digests with added <sup>15</sup>NH<sub>4</sub>:** Greater than 90% of NH<sub>4</sub> in most digests had been recovered on the disks by the end of the last diffusion period (Table 5), with recoveries being slightly lower for digests containing >450 µg N. Matrix effects due to soil type and organic amendment appear to be secondary to the effect of N quantity. Recovery was inversely related to the quantity of N at any given time for all soils particularly for values >400 µg N. Despite very low total recoveries particularly at the earlier sampling times, the observed <sup>15</sup>N atom abundance of the diffused NH<sub>3</sub> approximated the expected values (Table 6). As a percentage of the expected <sup>15</sup>N value, the observed values were >96% and only at the first sampling time (6 h) were there some values < 95%.

Table 4: Nitrogen recovered as Total NH<sub>4</sub> from direct soil-HF/HCl digest amended with organic N using optimal KOH specification for distillation

Soil series	Organic N amendment	Maximal N in sample <sup>a</sup>	Diffusion Period				
			6 h	48 h	96 h	144 h	192 h
			(µg)				
Piarco	Native	34.5	0.00 (0.00) <sup>b</sup>	17.1 (1.39)	25.70 (1.22)	32.9 (2.68)	36.9 (1.58)
	Rice Husks	59.3	0.00 (0.00)	18.6 (2.06)	35.70 (2.65)	56.9 (0.99)	59.2 (1.46)
	Manure	80.4	21.70 (3.89)	56.7 (2.05)	73.82 (0.71)	85.1 (1.54)	84.6 (1.46)
	Compost	271	43.50 (1.10)	161.0 (2.65)	204.00 (1.42)	276.0 (2.12)	279.0 (1.41)
	Glycine	34.5	0.00 (0.00)	18.6 (0.99)	29.30 (3.54)	33.4 (1.18)	37.1 (0.50)
Bejucal	NH <sub>4</sub> -N	235	45.00 (2.33)	124.0 (0.00)	185.00 (1.24)	231.0 (2.53)	237.0 (2.12)
	Native	238	57.20 (1.27)	113.0 (0.97)	176.00 (1.33)	224.0 (1.13)	229.0 (1.40)
	Rice Husks	263	63.60 (0.49)	118.0 (2.19)	190.00 (1.41)	259.0 (2.23)	259.0 (0.07)
	Manure	284	75.30 (1.52)	152.0 (0.71)	212.00 (1.65)	275.0 (1.12)	286.0 (1.96)
	Compost	474	147.00 (2.12)	270.0 (1.41)	333.00 (2.05)	465.0 (2.24)	493.0 (1.33)
Princes Town	Glycine	238	74.50 (1.06)	155.0 (2.12)	196.00 (3.53)	248.0 (2.31)	244.0 (1.10)
	NH <sub>4</sub> -N	438	99.30 (1.91)	174.0 (1.41)	303.00 (0.71)	420.0 (2.02)	429.0 (3.00)
	Native	119	17.10 (2.97)	55.8 (0.28)	82.30 (1.92)	115.0 (1.32)	122.0 (0.84)
	Rice Husks	144	18.60 (0.00)	62.1 (1.10)	107.00 (1.09)	140.0 (2.19)	146.0 (1.55)
	Manure	165	54.30 (0.46)	99.3 (3.47)	140.00 (1.41)	168.0 (2.12)	178.0 (2.08)
Montserrat	Compost	355	137.00 (2.00)	203.0 (1.16)	276.00 (2.52)	345.0 (1.58)	369.0 (1.00)
	Glycine	119	26.60 (1.68)	67.2 (2.37)	98.90 (3.04)	118.0 (1.79)	121.0 (1.58)
	NH <sub>4</sub> -N	319	115.00 (0.51)	151.0 (1.47)	211.00 (1.49)	304.0 (0.24)	324.0 (1.65)
	Native	84.3	0.00 (0.00)	32.6 (0.00)	62.10 (0.26)	77.6 (0.00)	80.4 (0.77)
	Rice Husks	109	16.40 (1.30)	45.0 (1.57)	75.00 (1.41)	96.6 (0.00)	102.0 (0.50)
River Estate	Manure	130	24.80 (2.37)	74.5 (1.58)	102.00 (0.46)	124.0 (1.38)	129.0 (1.24)
	Compost	320	52.80 (1.24)	154.0 (0.90)	234.00 (1.29)	313.0 (1.13)	320.0 (1.61)
	Glycine	84.3	0.00 (0.00)	20.6 (0.00)	57.40 (0.90)	77.6 (1.13)	82.3 (1.40)
	NH <sub>4</sub> -N	284	41.90 (0.50)	140.0 (1.52)	219.00 (1.61)	296.0 (1.40)	276.0 (0.90)
	Native	165	33.70 (1.15)	87.9 (1.33)	126.00 (1.49)	165.0 (2.05)	169.0 (0.10)
Caroni	Rice Husks	190	36.20 (1.26)	97.8 (2.41)	128.00 (0.00)	187.0 (1.99)	189.0 (2.17)
	Manure	211	52.10 (0.90)	109.0 (2.56)	186.00 (1.34)	213.0 (0.23)	226.0 (2.68)
	Compost	401	104.00 (0.86)	191.0 (2.11)	286.00 (0.42)	391.0 (1.89)	414.0 (1.64)
	Glycine	165	42.10 (1.44)	95.5 (2.55)	143.00 (2.06)	167.0 (1.24)	175.0 (.097)
	NH <sub>4</sub> -N	365	99.40 (2.14)	182.0 (1.45)	2.45 (1.63)	329.0 (1.15)	363.0 (0.92)
Chaguaramas	Native	282	93.10 (1.09)	159.0 (0.97)	241.00 (3.04)	279.0 (1.79)	297.0 (1.81)
	Rice Husks	307	109.00 (1.39)	192.0 (1.03)	250.00 (2.25)	301.0 (2.50)	320.0 (1.92)
	Manure	328	110.00 (1.99)	199.0 (2.20)	268.00 (1.10)	320.0 (0.88)	345.0 (1.08)
	Compost	518	155.00 (1.36)	244.0 (1.01)	386.00 (1.26)	507.0 (1.12)	542.0 (0.77)
	Glycine	282	103.00 (1.38)	199.0 (2.19)	255.00 (1.53)	290.0 (1.55)	286.0 (1.24)
Chaguaramas	NH <sub>4</sub> -N	482	180.00 (3.01)	292.0 (2.18)	388.00 (1.28)	475.0 (0.56)	490.0 (1.03)
	Native	174	55.90 (0.00)	93.3 (1.92)	133.00 (2.17)	161.0 (1.33)	173.0 (2.31)
	Rice Husks	199	69.80 (1.60)	132.0 (2.48)	185.00 (2.14)	201.0 (1.32)	214.0 (1.70)
	Manure	220	71.40 (1.41)	134.0 (0.87)	196.00 (1.34)	212.0 (1.25)	226.0 (0.44)
	Compost	410	129.00 (1.06)	206.0 (1.29)	319.00 (1.46)	416.0 (2.19)	439.0 (1.15)
Chaguaramas	Glycine	174	69.80 (1.29)	102.0 (1.24)	151.00 (1.95)	157.0 (2.33)	179.0 (1.70)
	NH <sub>4</sub> -N	374	161.00 (0.52)	259.0 (1.88)	332.00 (0.59)	370.0 (1.21)	388.0 (1.03)

<sup>a</sup> Maximal N refers to the total quantity of available NH<sub>4</sub> after HF digestion determined via distillation plus the quantity of 2 M KCl extractable NH<sub>4</sub> in added materials: Rice husks - 24.8 µg, Manure - 45.9 µg, Compost - 240 µg, Glycine - 0 µg, NH<sub>4</sub>-N - 200µg

<sup>b</sup> Standard Deviations in parentheses

## DISCUSSION

**Distillation of soil-acid digests spiked with organic and inorganic N:** Minimal and maximal recoveries of labile organic and spiked inorganic N provides strong evidence that little, if any organic N is liberated as NH<sub>4</sub> during distillation with 32.5 mL of 2 M KOH. Accordingly, this KOH specification would allow accurate determination of

dissolved NH<sub>4</sub> in the soil-acid digest. Quantities of labile organic N most likely to convert to NH<sub>4</sub> and liberate during distillation are present at much higher levels in the amendments than in the soil. Accordingly, the small quantities that were recovered from these amendments at the optimum KOH specification suggest that even smaller quantities of any labile organic N associated with soil should be recovered.

Table 5: Quantitative recovery of NH<sub>4</sub> on filter disks from direct soil-HF/HCl digest using optimal KOH specification for distillation

Soil series	Maximal N in sample <sup>a</sup> µg	Diffusion Period				
		6 h	48 h	96 h	144 h	192 h
Piarco	135	24.5 (6.65) <sup>b</sup>	41.4 (0.67)	61.6 (4.09)	89.7 (0.95)	98.2 (2.80)
Bejucal	338	19.3 (1.81)	45.8 (1.85)	66.0 (1.28)	87.4 (2.25)	94.5 (2.54)
Princes Town	219	24.3 (0.33)	40.5 (3.65)	61.2 (4.72)	83.6 (2.11)	96.8 (1.53)
Montserrat	184	21.2 (4.67)	48.3 (1.16)	68.3 (3.92)	87.9 (1.76)	95.1 (1.55)
River Estate	265	20.8 (4.42)	43.3 (1.64)	68.9 (2.45)	85.4 (1.97)	94.4 (3.29)
Caroni	382	15.6 (5.71)	36.6 (3.69)	49.8 (3.21)	65.4 (3.12)	91.3 (0.26)
Chaguaramas	274	20.5 (3.12)	46.7 (6.66)	60.5 (2.69)	84.5 (0.49)	95.7 (1.70)

<sup>a</sup> Maximal N refers to the total quantity of available NH<sub>4</sub> after HF digestion plus 100 µg of N as (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

<sup>b</sup> Coefficients of Variation in parentheses

Table 6: Recovery of <sup>15</sup>N from 10 mL spiked (100 µg <sup>15</sup>NH<sub>4</sub>-N) direct soil-HF/HCl digests of a range of soils amended with organic N along with expected and observed <sup>15</sup>N atom abundance

Soil Series	Expected <sup>15</sup> N in Sample <sup>a</sup>	Diffusion Period									
		6 h		48 h		96 h		144 h		192 h	
		O <sup>15</sup> N <sup>b</sup> (% a. a.) <sup>d</sup>	<sup>15</sup> N <sup>c</sup> (%)	O <sup>15</sup> N (% a. a.)	<sup>15</sup> N (%)	O <sup>15</sup> N (% a. a.)	<sup>15</sup> N (%)	O <sup>15</sup> N (% a. a.)	<sup>15</sup> N (%)	O <sup>15</sup> N (% a. a.)	<sup>15</sup> N (%)
Piarco	7.95	7.32	92.2 (0.02) <sup>c</sup>	7.75	97.5 (0.09)	7.82	98.4 (0.03)	7.80	98.1 (0.07)	7.78	97.9 (0.08)
Bejucal	3.39	3.31	97.5 (0.02)	3.33	98.3 (0.05)	3.34	98.6 (0.04)	3.37	99.5 (0.01)	3.38	99.8 (0.04)
Princes town	5.04	4.75	94.2 (0.01)	4.91	97.5 (0.04)	5.00	99.3 (0.09)	4.92	97.7 (0.05)	4.95	98.2 (0.03)
Montserrat	5.93	5.73	96.6 (0.02)	5.76	97.1 (0.01)	5.86	98.8 (0.03)	5.86	98.9 (0.02)	5.88	99.1 (0.02)
River Estate	4.23	4.08	96.5 (0.02)	4.14	97.8 (0.02)	4.20	99.2 (0.01)	4.21	99.6 (0.01)	4.20	99.4 (0.02)
Caroni	3.05	2.91	95.3 (0.01)	2.94	96.3 (0.05)	2.95	96.7 (0.02)	3.00	98.3 (0.01)	2.99	98.0 (0.02)
Chaguaramas	4.10	3.94	96.1 (0.04)	4.00	97.5 (0.02)	4.05	98.7 (0.01)	4.01	97.8 (0.01)	4.08	99.5 (0.02)

Expected <sup>15</sup>N atom abundance calculated based on the mass balance equation shown in the methodology; Observed <sup>15</sup>N atom abundance from ANCA-MS; Recovery of <sup>15</sup>N measured as the observed <sup>15</sup>N as a percentage of the expected, Percentage atom abundance; Coefficients of variation in parentheses

Labile soil organic N is determined by distillation of soil-acid hydrolysates after various pre-treatments of NaOH, ninhydrin (C<sub>9</sub>H<sub>6</sub>O<sub>4</sub>) and phosphate-buffer<sup>[15]</sup> depending on whether amino acid- or amino sugar-N is being determined. The distillations are done by slowly adding adequate amounts of 10 M NaOH to the distillation flask, collecting the liberated NH<sub>3</sub> in boric acid-indicator solution and determining concentrations by back titration with dilute H<sub>2</sub>SO<sub>4</sub>. Using MgO instead of NaOH in the distillation of these hydrolysates gives only NH<sub>4e</sub> in the soil and by difference with the 10 M NaOH distillations the labile N fractions can be calculated. Dependent on organic N pool, large quantities of labile organic N can be hydrolyzed during distillation, particularly with concentrated alkali. Ammonia released from amino acids<sup>[15]</sup> occurs from their reaction with ninhydrin and is unlikely to be hydrolyzed with alkali, suggesting that amino-sugars and more easily hydrolysable fractions were the sources of alkali-labile N in the amendments.

Paramasivam, S. and G.A. Breitenbeck<sup>[4]</sup> indicated that the volume of 2 M KOH needed to accurately measure the quantity of NH<sub>4</sub> by membrane diffusion should just increase the pH of the digest to 9 to minimize

any hydrolysis of organic N by alkali. In our studies, however, the optimum pH was 13.4. Since distillations last just about three minutes compared to much longer times for membrane diffusions, a higher pH is needed to ensure complete diffusion of NH<sub>4</sub> during the short distillation period.

**Microdiffusion study of organically amended direct soil-acid digests:** Eudoxie, G.D. and G.A. Gouveia<sup>[6]</sup> observed non-significant effects of N quantity on diffusion period, which could have been masked (in their experiments) by the stronger effect of volume. In these incubations, volume was constant, which might have resulted in a recognizable quantity effect. Dependent on initial quantity, >100 µg of NH<sub>4</sub>-N was diffused after 48 h and in a few cases 6 h, indicating the importance of N quantity in determining minimal diffusion period. Similar recoveries of NH<sub>4</sub>-N from organic amendments were reported via distillation (Table 2) indicating that diffusion procedures are just as effective in quantifying total NH<sub>4</sub>-N and do not result in recovery of significant organic N, using the optimal volume and molarity of KOH. Slight (<1%) recovery<sup>[4]</sup> of labile organic N during quantification of total NH<sub>4f</sub> did not significantly influence



total  $\text{NH}_4$  quantities. implying that the procedure can accurately quantify free inorganic  $\text{NH}_4$  present in the digest.

**Microdiffusion of soil-acid digests with added  $^{15}\text{NH}_4$ :** Similar diffusion periods used by<sup>[6]</sup> gave lower recoveries on acidified disks than in boric acid and the authors alluded to the smaller surface area of the disks. In another study<sup>[6]</sup> using 5 M HF 1 M HCl digests<sup>[5]</sup> with KOB<sub>r</sub> pre-treatment, >96% of the maximal-N was recovered at the first diffusion period. In those studies, the quantity of N was smaller (< 400  $\mu\text{g}$  N). For N quantities significantly >400  $\mu\text{g}$ , absorption capacities of the acidified glass-fibre traps may be retarding the rate of diffusion. The slower rate of adsorption by acidified glass-fibre discs, however, should be of little concern since microdiffusion before  $^{15}\text{N}$  analysis requires the pre-concentration of < 100  $\mu\text{g}$  N.

The data presented here suggest that the digest prepared after washing the soil with KOB<sub>r</sub>-KOH has certain characteristics that may promote faster diffusion of  $\text{NH}_4$ <sup>[6]</sup>. Additionally, use of a lower concentration of KOH combined with a higher volume that would have reduced the exposed surface area to volume ratio, may also have reduced the diffusion rate as well. The fact that the observed and expected  $^{15}\text{N}$  values were in agreement suggests that organic labile fractions do not diffuse during incubation. Even in the early stages of incubation where after 6 h only 8.9 - 24.5% of the total  $\text{NH}_4$  in the digest had been diffused and trapped on the acidified disks, there was little evidence of isotope fractionation of N.

### CONCLUSION

The analytical techniques reported herein highlights modifications to existing distillation and microdiffusion procedures for accurately determining total  $\text{NH}_4$ . The optimal concentration of 32.5 mL 2 M KOH resulted in quantitative recovery of  $\text{NH}_4$ -N in direct HF/HCl digests with minimal recovery of labile organic N compounds. This methodology provides a fast, low-input strategy for quantification and  $^{15}\text{N}$  pre-concentration of these N fractions.

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