Mineralizable Soil Nitrogen: Amounts and Extractability Ratios[1]

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ABSTRACT

Studies were conducted on a ^{15}N-labeled Weirdale loam, a Dark Gray Chernozemic soil (Boralfic Boroll) to (i) determine the amounts of N released by several methods previously used to obtain an estimate of potentially mineralizable N, (ii) determine their ^{15}N enrichment and extractability ratios, and (iii) compare the results from the above with the N mineralized during incubation and NH_4^+ released by the chloroform fumigation incubation technique. The NH_4^+-N accumulated during 10 d in fumigated soils accounted for ~1% of total N, was highly labeled, and had extractability ratios of 6.6 to 7.4. These ratios were similar to ones obtained for N mineralized during incubation of unfumigated soils. Ammonium-N extracted with dilute acidic permanganate solution ($0.01M$ $KMnO_4$ in 0.1 or $0.5M$ H_2SO_4) accounted for 0.72 to 0.84% of total N and had extractability ratios ranging from 3.4 to 3.9. A stronger solution of acidic permanganate extracted more N that was less enriched. Dilute sulfuric acid extracted NH_4^+ and organic N that had extractability ratios of < 3. Ammonium-N released by autoclaving the soil accounted for ~1% of total N and had extractability ratios ranging from 0.6 to 0.9. Acid hydrolysis showed that 72% of total N was hydrolyzable, 32% was amino acid–N and 20% was NH_4^+ released on hydrolysis. The extractability ratio for NH_4^+ released on hydrolysis was 1.7 and was significantly ($P < 0.01$) greater than extractability ratios of hydrolyzable N and amino acid–N. The similarity and high extractability ratios of NH_4^+ released after fumigation and NO_3^--N accumulating during aerobic incubation indicated that the fumigation extracted a biologically meaningful fraction. The biomass was responsible for only 15 to 25% of the net N mineralized during a 12-week incubation. Results indicated that (i) extraction of a highly labeled N pool in soil can only partly explain the source of N being mineralized, (ii) N is mineralized from several pools, and (iii) there is a remote possibility that a single extractant can extract the variety of N compounds undergoing mineralization and immobilization in soil.

Additional Index Words: N_o, N supplying power, N availability ratios, active N phase, biomass N, nonbiomass active N, total N.

THE AMOUNT AND ISOTOPIC RATIO of organic C and N compounds extracted from soil by various techniques can yield useful information on organic compounds undergoing mineralization. Comparison of ^{14}C or ^{15}N enrichment of extracted compounds with that of CO_2-C or NO_3^--N mineralized during incubation may indicate whether biologically meaningful fractions are extracted. Jenkinson (1968) showed that $^{14}CO_2$-C evolved from a fumigated soil had a higher specific activity compared with C extracted with cold $0.1M$ HCl, boiling saturated $CaSO_4$ solution, or $0.05M$ $Ba(OH)_2$. Also, reagents that were strongly hydrolytic or those that extracted humic materials yielded fractions that were relatively lightly labeled.

Chichester (1970) physically fractionated a ^{15}N-labeled soil, extracted the particle-size fractions with hot, dilute sodium pyrophosphate, and determined the atom % excess of N compounds in the extract after acid hydrolysis. The enrichment of various N forms in all the particle-size fractions was lower than the enrichment of N mineralized during a 4-week incubation of the particle-size fractions. Legg et al. (1971) autoclaved a ^{15}N-labeled soil and found that the ^{15}N enrichment of amino acids, amino sugars, and unidentified N in the $CaCl_2$ extracts was lower than the enrichment of total N in plants grown in the same soil under greenhouse conditions. This implied that the selectivity of the extractants was poor from the standpoint of biologically available N. In contrast, the NH_4^+ accumulated during a 10-d incubation of a fumigated soil had a similar ^{15}N enrichment compared with the NO_3^--N accumulated during incubation (Amato and Ladd, 1980; Paul and Juma, 1981). The enrichment of these fractions was much higher than that of total N in soil. These observations are consistent with those of Jenkinson (1968) and show that the microbial biomass is a source and sink for C and N as well as part of the active C and N phase in soil.

Stanford and Smith (1978) found that the NH_4^+ released by partial oxidation of soil organic matter with dilute acidic permanganate solutions accounted for approximately one third to one half of the potentially mineralizable nitrogen, N_o. On the basis of the high correlation between the amount of NH_4^+ extracted and the N_o of 62 soils, they concluded that the extracted NH_4^+ came from a soil fraction readily susceptible to biological mineralization. Further work is necessary to elucidate the source and nature of N compounds released during the various extraction techniques that try to mimic the action of microbial enzymes in soil.

The objectives of this study are (i) to determine the amount and isotopic ratio of N compounds extracted, with dilute $CaCl_2$ after autoclaving the soil, with dilute acid and acidic permanganate solution, and after $6M$ HCl hydrolysis; and (ii) to compare the ^{15}N enrichment of NO_3^--N and biomass N with those of extracted N.

MATERIALS AND METHODS

Incubation Experiments

The ^{15}N enriched surface soil samples of a Weirdale loam, a Dark Gray Chernozem (Boralfic Boroll), were obtained in fall from a field trial. This had investigated the effects of aqua NH_3 and urea, in the presence and absence of a nitrification inhibitor [4-amino-1,2,4-triazole, (ATC)] on N distribution after a wheat crop (Juma and Paul, 1983). The soil with a total N content of 0.44% and a pH of 7.4 (1:2.5 water suspension) was incubated at 34 kPa of soil pore moisture tension and at $28 \pm 1°C$ to obtain CO_2 and NO_3-N production during net mineralization.

Analysis

Nitrate N During Incubation—The leaching incubation technique (Stanford and Smith, 1972) was used to determine

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N mineralized during incubation. The atom % ^{15}N abundance of the mineral N leached was determined.

Ammonium N Extracted by Chloroform Fumigation Incubation Technique—Duplicate, 50-g moist soil samples (ODB) were fumigated with chloroform for 24 h in a desiccator, followed by evacuation of these vapors and subsequent incubation for 10 d at 28 ± 1°C. Ammonium-N was extracted with 2M KCl and its atom % ^{15}N abundance was determined.

$$\text{Biomass N} = (\text{NH}_4^+\text{-N extracted after 10 d})/k_N$$

where k_N was 0.28 (Paul and Voroney, 1980).

Extraction of Soil N by Autoclaving in Dilute CaCl$_2$—Ten grams of ^{15}N enriched soil from laboratory incubation experiments sampled at the start of the experiment were placed in each of the two 50-mL polypropylene centrifuge tubes, and 25 mL of dilute CaCl$_2$ (0.01M) was added. The samples were autoclaved for 16 h, cooled, and centrifuged (Stanford, 1968). The extract was saved, and the residue was washed twice with 25-mL aliquots of dilute CaCl$_2$. All extracts of each sample were pooled, and the volume was made up to 100 mL. Two 20-mL aliquots from the composite extract were anlayzed for NH$_4^+$-N and its ^{15}N abundance.

Extraction of Soil N with Acid and Acidic Permanganate Solutions—The finely ground (< 100-mesh particle size) soil samples (2 X 1 g) from NH$_4$OH+ATC treatment were extracted with 25 mL H$_2$SO$_4$ at 0.025, 0.05, 0.10, 0.25, and 0.5M (Stanford and Smith, 1978). Duplicate, 5 mL-aliquots were analyzed for NH$_4^+$ and total N. The ^{15}N abundance was measured on the composite of four distillates. Organic N and its ^{15}N enrichment was determined by difference between the above-mentioned analyses.

Acidic permanganate extractions were carried out on soil samples that were preextracted with either 0.1 or 0.5M H$_2$SO$_4$. The residue was first washed with 25 mL of deionized water and subjected to the following treatments:

1. The finely ground soil samples (2 X 1 g) were extracted with 25 mL of acidic permanganate solutions made in 0.5M H$_2$SO$_4$.
2. The finely ground soil samples were extracted with 25-mL acidic permanganate solutions made in 0.1M H$_2$SO$_4$.
3. The coarser samples (< 2-mm particle size) were extracted with acidic permanganate solutions made in 0.5M H$_2$SO$_4$.

The final KMnO$_4$ solution strength was 0.01, 0.02, 0.04, 0.10, and 0.20M in all cases. Duplicate 5-mL aliquots of extracts were anlayzed for NH$_4^+$-N. The atom % ^{15}N abundance was measured on the composite of four distillates.

Extraction of Soil N after Acid Hydrolysis—The different forms of soil N were determined as described below and their atom % ^{15}N abundance was determined.

Total Hydrolyzable N—Total hydrolyzable N was determined by the method of Bremner (1965). Two 10-g freeze-dried, ground (< 100-mesh) soil samples were hydrolyzed with 6M HCl (100 mL) for 16 h under reflux. The mixture was filtered and the volume of hydrolyzate was made up to 200 mL. A 100-mL aliquot was neutralized to pH 6.5 ± 0.1 and the volume made to 200 mL. Two 20-mL aliquots were used for total N determination (Rennie and Paul, 1971).

Amino acid-N—Two 20-mL aliquots of neutralized hydrolyzate from each sample were used to determine the amino acid-N by the ninhydrin-ammonia method (Bremner, 1965).

Ammonium-N Released by 6M HCl Hydrolysis—Two 20-mL aliquots of neutralized hydrolyzate from each sample were used to determine NH$_4^+$-N by steam distillation with MgO powder (Bremner, 1965).

Preparation of Samples for ^{15}N Determination—Steam distillation was used in all N analyses. The distillate was collected in boric acid and titrated to pH 4.8 with 0.025 or

0.05M H$_2$SO$_4$. The titrated sample was acidified (0.2 mL of 0.025M H$_2$SO$_4$) and concentrated to dryness in a sample evaporator (Brinkmann sample concentrator SC/24R; temperature set at 50°C). If the sample had < 1 mg of N, it was spiked with 1 mL (1 mg mL^{-1}) of analytical-grade (NH$_4$)$_2$SO$_4$-N of known atom % abundance before concentrating the sample. Approximately 1 mL of deionized water was used to redissolve the salts. The samples were transferred with disposable glass Pasteur pipettes into 15 by 85 mm disposable culture glass tubes and dried at 90°C. The tubes were capped with disposable tops for autoanalyzer vials. Analyses for ^{15}N atom % abundance were carried out on either an Atlas GD150 or Micromass 602E mass spectrometer.

Precision and Reproducibility of ^{15}N Analysis—The ^{15}N analysis of standard (NH$_4$)$_2$SO$_4$ showed that the results were reproducible with a standard deviation of ± 0.00003 atom % ^{15}N abundance. The standard deviation of atom % ^{15}N of total N in the NH$_4$OH treated Weirdale loam was 20-fold greater (0.37601 ± 0.0006, $n=8$). This included the machine error as well as errors during Kjeldahl digestion, distillation, and sample preparation. Work in our laboratory has shown that there is more variability in amount of N measured as total N or mineral N compared with ^{15}N analysis.

RESULTS

The Weirdale loam used in this investigation was obtained from one of the 20 sites in which ^{15}N-labeled fertilizer trials were carried out, and its behavior with respect to ^{15}N dynamics was typical to the others (Paul and Rennie, 1977; Juma and Paul, 1983). The ^{15}N labeled samples were treated by a number of extraction methods previously used in attempts to chemically define N$_o$. The atom % ^{15}N excess of N compounds extracted by various methods can be compared directly or by using the extractability ratio (Legg et al., 1971):

$$\text{Extractability ratio} = \frac{(\text{Tagged N extracted}) \div (\text{Total N extracted})}{(\text{Tagged total N}) \div (\text{Total N})}.$$

This simplifies to

$$\text{Extractability ratio} = \frac{(\text{atom \% }^{15}\text{N excess of extracted N})}{(\text{atom \% }^{15}\text{N excess of total N})}.$$

The lower limit of extractability ratios is zero and would be obtained if no labeled N was extracted. An extractability ratio > 1 implies that N compounds extracted are relatively enriched in ^{15}N compared with enrichment of total N in soil.

Effect of Autoclaving

The NH$_4^+$-N extracted by autoclaving soils ranged from 49 to 66 µg g^{-1} of soil and accounted for 0.98 to 1.44% of total N (Table 1). The amount of NH$_4^+$-N extracted in the urea + ATC treatment was significantly lower ($P < 0.01$) compared with other treatments. The total N in the different treatments varied as the soil was sampled by depth of the A horizon. The atom % excess of composite samples of four distillates ranged from 0.0056 to 0.0070 and was lower than that of total N in soils. Thus, the extractability

Table 1—Amounts and extractability ratios of NH_4^+-N extracted by autoclaving Weirdale loam soil.

Treatment	$\mu g\ g^{-1}$ soil	NH₄⁺-N in CaCl₂ extracts % of total N	atom % ¹⁵N excess†	Extractability ratio‡
NH₄OH	63 a§	1.44	0.0059	0.9
NH₄OH + ATC	66 a	1.30	0.0056	0.6
Urea + ATC	49 b	0.98	0.0070	0.8

† The composite of four distillates of each treatment was analyzed for ¹⁵N enrichment.
‡ The atom % ¹⁵N excess of total N in NH₄OH-, NH₄OH + ATC-, and urea + ATC-treated Weirdale loam were 0.0065, 0.0098, and 0.0091, respectively.
§ Group means were compared with Scheffe test. Group means followed by the same letter are not significantly different at $P < 0.05$.

ratios ranged from 0.6 to 0.9 (Table 1). More unlabeled NH_4^+-N was extracted by autoclaving the soil compared with labeled N, which shows that this method does not selectively release NH_4^+ from ¹⁵N-labeled organic N.

Extraction with Dilute Sulfuric Acid

The amount of NH_4^+-N extracted increased from 19 to 39 $\mu g\ g^{-1}$ as the sulfuric acid concentration increased from 0.025 to 0.50M (Table 2). This accounted for 0.38 to 0.76% of the total N in soil. The extractability ratios of NH_4^+ ranged from 0.6 to 0.7. The amount of NH_4^+-N extracted (Y) by different concentrations (X) of sulfuric acid could be described by the hyperbolic equation

$$Y = 40(\pm 2) \cdot X/[0.040(\pm 0.007) + X].\quad [1]$$

The figures in parentheses are standard errors of the parameter estimates, RMS $= 13.7\ \mu g\ g^{-1}$ of soil with 16 df.

The organic N extracted (Y) ranged from 73 to 134 μg of N g^{-1} of soil as the concentration (X) of sulfuric acid increased (Table 2) and could be described as follows:

$$Y = 135(\pm 5) \cdot X/[0.020(\pm 0.003) + X].\quad [2]$$

The figures in parentheses are standard errors of parameter estimates; RMS $= 9.6\ \mu g\ g^{-1}$ of soil with 14 df. The organic N extracted accounted for 1.43 to 2.64% of total N. The extractability ratios of organic N decreased as the concentration of acid increased. Also, the amounts and extractability ratios of organic N extracted were greater than NH_4^+-N extracted with dilute acid.

Extraction with Acidic Permanganate

The Weirdale loam, after extraction with 0.1 or 0.5M H_2SO_4, was washed with water and extracted with acidic permanganate solution. The NH_4^+ released due to partial oxidation was measured and its atom % ¹⁵N abundance was determined. The amount of NH_4^+ released increased as the strength of $KMnO_4$ increased. For example, the amount of NH_4^+-N released increased from 42 $\mu g\ g^{-1}$ at 0.5M H_2SO_4 plus 0.01M $KMnO_4$, to 482 $\mu g\ g^{-1}$ at 0.5M H_2SO_4 plus 0.2M $KMnO_4$, and accounted for 9.5% of total soil N (Table 3). The additional NH_4^+ extracted (Y) was related to the strength of $KMnO_4$ (X) as follows:

$$Y = 920(\pm 40) \cdot X/[0.186(\pm 0.014) + X].\quad [3]$$

Table 2—Effect of sulfuric acid concentration on the amounts and extractability ratios of NH_4^+-N and organic N extracted from Weirdale loam soil.

Acid concentration, M	Extracted N $\mu g\ g^{-1}$ soil	% of total N	atom % ¹⁵N excess†	Extractability ratio
	NH₄⁺-N			
0.025	19	0.38	0.0062	0.6
0.05	22	0.44	0.0063	0.7
0.10	29	0.57	0.0061	0.6
0.25	33	0.64	0.0057	0.6
0.50	39	0.76	0.0071	0.7
	Organic N			
0.025	73	1.43	0.0210	2.1
0.05	109	2.16	0.0135	1.4
0.10	105	2.07	0.0151	1.5
0.25	125	2.46	0.0100	1.0
0.50	134	2.64	0.0118	1.2

† Atom % ¹⁵N excess of the composite of four distillates.

The figures in parentheses are standard error estimates of the parameters; RMS $= 10.9\ \mu g\ g^{-1}$ of soil with 18 df. The extractability ratio of NH_4^+-N decreased with increased $KMnO_4$ concentration. However, the extractability ratio of 3.9 was obtained when the concentration of acidic permanganate was low. Thus, mild acidic $KMnO_4$ solution released relatively highly labeled NH_4^+-N compared with stronger $KMnO_4$ solutions.

In the second series of experiments, the oxidation potential of acidic $KMnO_4$ solutions was decreased by decreasing the strength of H_2SO_4 from 0.5 to 0.1M. The weaker oxidizing solution decreased the amount of NH_4^+-N extracted and up to 7.5% of total N was extracted (Table 3). The data could not be fitted to the hyperbolic equation. The extractability ratios of NH_4^+ decreased from 3.4 to 1.0 as the $KMnO_4$ concentration increased. Thus, mild acidic permanganate solutions extracted highly labeled NH_4^+-N; however, regression analyses showed that extractability ratios were not significantly related to the concentration of the acidic permanganate.

Table 3—Effect of oxidation potential and soil particle size on additional NH_4^+-N extracted and its extractability ratio from Weirdale loam soil.

H_2SO_4, M	KMnO₄ conc, M	Additional NH₄⁺-N extracted†, $\mu g\ g^{-1}$ soil	Additional NH₄⁺-N extracted/ total N, %	Atom % ¹⁵N excess‡	Extractability ratio
		<100-mesh soil particle size			
0.5	0.01	42	0.84	0.0383	3.9
0.5	0.04	174	3.42	0.0090	0.9
0.5	0.20	482	9.49	0.0037	0.4
		<100-mesh soil particle size			
0.1	0.01	37	0.72	0.0334	3.4
0.1	0.04	90	1.77	0.0182	1.9
0.1	0.20	377	7.42	0.0094	1.0
		<2-mm soil particle size			
0.5	0.01	28	0.55	0.0240	2.5
0.5	0.04	164	3.22	0.0051	0.5
0.5	0.20	449	8.85	0.0030	0.3

† Initially, the soil samples were extracted with either 0.5 or 0.1M H_2SO_4. The residue was washed with water and reextracted with acidic permanganate solution. The data are reported additional NH_4^+-N extracted ($\mu g\ g^{-1}$ soil) due to acidic permanganate treatment.
‡ Atom of ¹⁵N excess of composite of four distillates.

Table 4—Amounts and extractability ratios of total hydrolyzable N, amino acid-N and NH_4^+ released on acid hydrolysis of Weirdale loam soil (NH_4OH treatment).

Form	Amount, μg N g^{-1} soil	% of total N	Atom % ^{15}N excess	Extractability ratio
Hydrolyzable N	3142 a†	72	0.0072 a	1.1
Amino acid N	1378 b	32	0.0059 a	0.9
NH_4^+ released on hydrolysis	876 c	20	0.0111 b	1.7
Total N	4368 d	100	0.0065 a	1.0

† Group means ($n = 4$) were compared with Scheffe test. Group means followed by the same letter are not significantly different at $P < 0.01$.

The soil particle size had little effect on the amount of NH_4^+-N extracted. The relationship between the amount of N extracted (Y) and concentration of acidic permanganate (X) was

$$Y = 959(\pm 135) \cdot X/[0.234(\pm 0.052) + X]. \quad [4]$$

RMS = 26.8 μg of N g^{-1} of soil with 18 df. Equation [4] is not significantly different from Eq. [3]. The extractability ratio of NH_4^+-N extracted from the soil that had passed through a 2-mm sieve was lower than from the soil that had passed through a 100-mesh sieve. This showed that increased amounts of labeled ^{15}N compounds were extracted in the finer soil compared with the coarser one. The similarity of the amounts of NH_4^+-N extracted suggests that the concentration of $KMnO_4$ was limiting the amount of NH_4^+-N extracted.

Acid Hydrolysis

About 72% of the total N was acid hydrolyzable (Table 4). Amino acid N accounted for 32% of total N, whereas NH_4^+-N released on hydrolysis accounted for 20%. The extractability ratio of NH_4^+ released on hydrolysis was significantly higher ($P < 0.01$) than that of hydrolyzable N and amino acid N. However, acid hydrolysis is a very drastic method to extract N compounds from soil and does not differentiate between N compounds present in the active organic phase from the passive N phase.

Chloroform Fumigation-Incubation

The atom % ^{15}N excess and the extractability ratios of NO_3^--N mineralized during the first 2 weeks and NH_4^+ extracted by the fumigation-incubation technique ranged from 6.1 to 8.2 for all the soils and were almost identical for each soil. Thus, the fumigation technique extracted a biologically meaningful active fraction. The extractability ratios of NO_3^--N produced during aerobic incubation and NH_4^+ released after fumigation and subsequent incubation declined slightly during incubation (data not shown). The biomass ^{14}N size calculated by using $k_N = 0.28$ (Paul and Voroney, 1980) decreased by 15 to 26 μg g^{-1} during a 12-week incubation. At the same time, NO_3^--N accumulated ranged from 95 to 102 μg g^{-1}. Thus, biomass N accounted for 15 to 25% of the net N mineralized.

DISCUSSION

Several different techniques were used in the present study to extract and measure the ^{15}N enrichment of N compounds in soil. The N mineralized and mea-

Table 5—Comparison of the amounts of NH_4^+ released by chloroform fumigation incubation technique and N mineralized during 2 weeks for Weirdale loam soil.

Treatment	N form			
	μg g^{-1} soil	% of total N	Atom % ^{15}N excess	Extractability ratio
NH_4^+ during 10-d incubation of fumigated soil				
NH_4OH	42 a†	0.96	0.043 a†	6.6
NH_4OH + ATC	50 b	0.98	0.068 b	7.4
Urea + ATC	51 b	1.03	0.067 b	7.4
N mineralized during 2 weeks in the laboratory				
NH_4OH	16 a‡	0.36	0.040§	6.1
NH_4OH + ATC	15 a	0.30	0.063	6.4
Urea + ATC	15 a	0.30	0.074	8.2

† Mean of four analyses. Group means were compared with Scheffe test. Means followed by the same letter are not significantly different at $P < 0.01$.
‡ Mean of six analyses.
§ Atom % ^{15}N excess of the composite of six distillates.

Table 6—Amounts and extractability ratios of various N fractions of Weirdale loam soil.

N form	N extracted/ total N, %	Extractability ratio
N mineralized during 2-week laboratory incubation	0.30–0.36	6.1–8.2
NH_4^+ extracted by Chloroform fumigation incubation technique	0.96–1.03	6.6–7.4
$0.01M$ $KMnO_4$ in $0.5M$ H_2SO_4	0.84	3.9
$0.01M$ $KMnO_4$ in $0.1M$ H_2SO_4	0.72	3.4
$0.025M$ H_2SO_4 extractable organic N	1.43	2.1
NH_4^+ released on acid hydrolysis	20	1.7
Hydrolyzable N	72	1.1
Amino acid N	32	0.9
NH_4^+ extracted by autoclaving	0.98–1.44	0.6–0.9

sured as NO_3^- during the laboratory incubation had the highest extractability ratios, ranging from 6.1 to 8.2 (Table 5). The NH_4^+ released by fumigating the soil had almost identical extractability ratios to NO_3^- and accounted for ~1% of the total N. If the factor of 0.28 is used, the biomass size in the Weirdale loam could account for almost 4% of total N. Jansson (1958) calculated the size of the active N phase using isotopic dilution techniques and found that it accounted for 10 to 15% of soil organic N. Paul and Juma (1981) expanded this concept and divided the active N phase into biomass and nonbiomass active N. The nonbiomass active N was 4% of the total N in the Weirdale loam.

Several extractants were used in an attempt to release portions of biomass or nonbiomass active N from soils. The NH_4^+ extracted with dilute acidic permanganate solution (after the initial acid extraction) accounted for 0.84% of the total N in soils and had extractability ratios that were almost one half of those of biomass or NO_3^--N (Table 6). The extractability ratios decreased as the oxidation potential increased (Table 3). Stanford and Smith (1978) observed that the additional NH_4^+ released by dilute acidic permanganate solution ($0.01M$ $KMnO_4$ in $0.5M$ H_2SO_4) accounted for 22% of the N_0 for 62 soils and suggested that this method should be used as a chemical index of N_0. The present investigation showed that this method indeed extracts a relatively enriched portion

that accounted for ~1% of soil organic matter. The initial acid extract released 39 μg of NH_4^+-N g^{-1} of soil and 134 μg of organic N g^{-1} of soil, whereas 42 μg of NH_4^+-N g^{-1} were extracted subsequently with dilute acidic permanganate solution. Dilute acid is known to lyse microbial cells and has been used to extract ATP from sediments (Lee et al., 1971). However, the dilute acid also extracted less labeled material, which caused the extractability ratio to be threefold to fourfold lower than that of biomass N (Table 6). The dilute acidic permanganate solution can possibly be used in a sequential extraction of organic N as it oxidizes and/or hydrolyzes a relatively ^{15}N enriched fraction.

Acid hydrolysis with 6M HCl is a drastic procedure that solubilizes a large portion of soil N. Hydrolyzable N accounted for 72% of soil N (Table 6). The extractability ratio of N compounds ranged from 0.9 to 1.7 and were similar to ones obtained by Chichester (1970) and Legg et al. (1971). Jenkinson (1968) also observed that the specific activity of ^{14}C decreased as more drastic methods were used to extract soil C. Thus, acid hydrolysis does not differentiate between active and recalcitrant N compounds in soil.

The NH_4^+ extracted by autoclaving the soil accounted for 1% of total N and had very low extractability ratios. Stanford and Smith (1976) found that the NH_4^+-N released by autoclaving the soil accounted for ~25% of N_o. Autoclaving kills the microbial biomass but the labeled N was not released as NH_4^+.

The atom % ^{15}N excess and extractability ratios of biomass N were much higher than any of the extractants used and were very similar to those of NO_3^--N produced during incubation (Table 6). The atom % ^{15}N excess of total N in the NH_4OH, $NH_4OH+ATC$, and urea+ATC soil samples at the beginning of the laboratory incubation was 0.0065, 0.0098, and 0.0091, respectively. ATC had caused a greater amount of fertilizer N to remain in organic and nonexchangeable NH_4^+ forms under field conditions (Juma and Paul, 1983). The atom % ^{15}N excess of biomass N and mineralized N in the NH_4OH treatment was 0.040 and 0.043, respectively, compared with 0.063 to 0.074 in the $NH_4OH+ATC$ and urea+ATC treatments (Table 5). Thus, biomass N and mineralized N reflected the effect of ATC. In contrast, the enrichment of NH_4^+-N extracted after autoclaving soil samples did not reflect the treatment effects.

The biomass was responsible for 15 to 25% of the net N mineralized during the 12-week incubation. These results imply that (i) extraction of a highly labeled N pool in soil can only partly explain the source of N mineralized, (ii) N is being mineralized from several pools, and (iii) there is only a remote possibility that a single extractant could extract the variety of N compounds undergoing mineralization and immobilization in soil. Further research should focus on organic N pools in soil and the use of tracer techniques in conjunction with new extraction techniques to aid in separating organic N into biologically meaningful fractions and in unraveling the mechanisms and controls of the mineralization-immobilization processes in soil.

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