EFFECT OF A NITRIFICATION INHIBITOR ON N IMMOBILIZATION AND RELEASE OF ^{15}N FROM NONEXCHANGEABLE AMMONIUM AND MICROBIAL BIOMASS

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The disposition of ^{15}N-aqua NH_3 and ^{15}N-solution urea in the presence and absence of a nitrification inhibitor [4-amino-1,2,4-triazole (ATC)] was measured under field conditions. ATC caused a 15% greater recovery of fertilizer N in the soil-plant system (95 vs. 80%) but no changes in wheat N uptake (37%). The 0- to 15-cm layer of ATC-treated soils contained 52–55% of the fertilizer N. The same layer of the non-ATC-treated soils contained 28–30%. The recovery of fertilizer N in the soil profile was 55–59% in ATC treatments compared to 40–42% in non-ATC treatments. Five to eight percent of fertilizer N was recovered in the non-exchangeable NH_4^+ fractions of A horizons of ATC-treated soils compared to ~1% in non-ATC treatments. Laboratory incubations and isotopic analysis of the ^{15}N-enriched soil, a Dark Gray Chernozem, showed that the nonexchangeable $^{15}NH_4^+$ was released at rates equivalent to a half-life of 38 wk (k = 0.018 wk^{-1}) at 28 ± 1°C and soil pore water potential of 34 kPa. Particle size and mineralogical analyses showed that the coarse clay fraction composed of mica, vermiculite and smectites contained 49% of the labeled nonexchangeable NH_4^+; the coarse silt fraction contained 26% of the labeled nonexchangeable NH_4^+. After growth of wheat fertilized with NH_4OH treated with ATC, the microbial biomass accounted for 41% of the organic ^{15}N remaining in soil. Soil samples from the ATC-treated plots contained almost two times the amount of ^{15}N in the microbial biomass compared to non-ATC treatments; this accounted for 46% of the organic ^{15}N remaining in the soil. The average half-life of microbial biomass ^{15}N was 27.6 wk in all the treatments. Thus, ATC caused a greater immobilization of fertilizer ^{15}N but no change in the rate of release of ^{15}N-microbial biomass. The conserved fertilizer would be slowly released over a long period of time.

Key words: Nitrogen immobilization, soil biomass, ^{15}N

[Effet d'un inhibiteur de nitrification sur l'immobilisation de N et sur la libération de N-15 à partir d'ammonium non échangeable et de la biomasse microbienne.]
Titre abrégé: Immobilisation et libération de N.
Le devenir de l'ammoniaque N-quinze et d'une solution d'urée N-quinze en présence d'un inhibiteur de nitrification (aminotriazole ou ATC) a été mesuré au champ. En présence d'ATC, 15 % plus du N de fumure ont été retrouvés dans le système sol-plante (95 % contre 80), mais il n'y a pas eu de changement pour l'absorption de N par le blé (37 %). La couche de 0 à 15 cm des sols traités par ATC contenait 52 à 55 % du N de fumure. Dans les sols non traités, la couche comparable n'en contenait que de 28 à 30 %. En tout, 55 à 59 % du N fertilisant était recouvrert dans le profil pédologique en présence d'ATC, contre 40 à 42 %

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en l'absence de l'inhibiteur. De 5 à 8 % du N a été récupéré dans les fractions de NH_4^+ non échangeable des horizons traités, contre approximativement 1 % dans les sols non traités. L'incubation en laboratoire et l'analyse par isotopes du sol enrichi de N-15, un chernozem gris foncé, ont révélé que le $^{15}NH_4^+$ non échangeable était libéré à des taux équivalant à une période biologique de 38 semaines (k = 0,018/semaine^{-1}) à la température de 28 °C ± 1 et à un potentiel hydrique du sol de 34 kPa. Les analyses granulométriques et minéralogiques ont montré que les fractions d'argile grossière, composées de mica, de vermiculite et de smectites, contenaient 49 % du NH_4^+ non échangeable, alors que la fraction limon grossier en contenait 26 %. Après culture de blé fertilisé par NH_4OH plus ATC, la biomasse microbienne représentait 41 % du N-15 organique restant dans le sol. Les échantillons de sol prélevés des parcelles traitées par ATC contenaient presque le double de N-15 dans la biomasse microbienne par comparaison aux parcelles non traitées. Cela représentait 46 % du N-15 organique restant dans le sol. La période biologique moyenne du N-15 de la biomasse microbienne était de 27,6 semaines dans tous les traitements. Ainsi, ATC a donné lieu à une plus grande immobilisation du N-15 fertilisant, mais n'a pas modifié le taux de libération du N-15 par la biomasse microbienne. La libération de l'engrais conservé par l'inhibiteur serait étalée sur une plus longue période de temps.

Mots clés: Immobilisation de l'azote, biomasse du sol, N-15

Numerous experiments, using isotopic and nonisotopic methods, have been conducted to test the effect of fertilizer carriers and time of application on the efficiency of fertilizer use. Generally, fall-applied N is less effective than spring-applied N (Frye 1978; Ridley 1977; Fried 1978; Nyborg and Leitch 1979) and losses of fall-applied NO_3^--N are higher than fall-applied urea or NH_4^+ (Paul and Rennie 1977).

The large losses of fall-applied NO_3^--N have sparked interest in the use of nitrification inhibitors to increase fertilizer use efficiency. Bundy and Bremner (1973) evaluated 24 nitrification inhibitors (NI) using laboratory incubations and concluded that N-serve [(2-chloro-6-trichloromethyl)pyridine] and ATC (4-amino-1,-2,4-triazole) were the most effective under laboratory conditions. The effectiveness of NI under field conditions depends upon the texture of the soil, rainfall and irrigation patterns, rooting depth of crops (Prasad et al. 1971) and the sensitivity of crops to NH_4^+ (Hendrickson et al. 1978).

A large portion of fertilizer N is transformed to slowly available forms during the growing season and up to 60% of fertilizer N can remain in the soil after a crop has been removed (Fried 1978). This fertilizer N could be present as nonexchangeable NH_4^+, organic N, or mineral N (NH_4^+, NO_2^- and NO_3^-). Black and Waring (1972) measured the changes in nonexchangeable NH_4^+-N levels in soils amended with NH_4^+ during two cropping cycles. They observed a relatively rapid decline in the initial rate of release of recently fixed NH_4^+; a slower decline in the release rate was found in the later stages. Kowalenko and Ross (1980) using ^{15}N tracer techniques noted that the recently fixed NH_4^+ in a fallow soil was released quickly during the first 3 mo (0.6 kg·ha^{-1}·day^{-1}) and at a much slower rate (~ 0.01 kg·ha^{-1}·day^{-1}) over the next 37 mo.

The use of NI can enchance the amount of fertilizer N that is immobilized because the persistence of NH_4^+ in the soil results in its incorporation into the active N phase. The release of immobilized fertilizer can be calculated by measuring the enrichment of NO_3^--N during the net mineralization phase (Jansson 1958) or by measuring the enrichment of total N in plants under cropped conditions (Legg et al. 1971). The microbial biomass is both a reservoir of the immobilized N and the agent for its mineralization. The measurement of microbial

biomass N in soil, using the chloroform-fumigation technique (Jenkinson and Powlson 1976) yields NH_4^+ which has an atom % ^{15}N abundance similar to NO_3^- produced during incubation, and makes it possible to better estimate the dynamics of recently incorporated N.

The objectives of this investigation were (1) to determine the fate of fertilizer N in the presence and the absence of a nitrification inhibitor (ATC) under field conditions, (2) to measure the release of non-exchangeable $^{15}NH_4^+$ under laboratory conditions, and (3) to measure the rate of ^{15}N release from the microbial biomass.

MATERIALS AND METHODS

Field Experiment

A field experiment to determine N uptake by plants and its distribution in soil was carried out on Weirdale loam, a Dark Gray Chernozemic soil. The surface horizon had a total N content of 0.43% and the pH was 7.4 (1:2.5 soil:water suspension).

Aluminum cylinders (20 cm i.d., 60 cm long) were used in N utilization studies involving ^{15}N-urea and ^{15}N-NH_4OH. The cylinders were located in macroplots that were used to determine the wheat yield response curve to different N carriers applied in the fall and in the spring. The treatments were solution urea and solution NH_3 with and without ATC (4-amino-1,2,4,-triazole hydrochloride) a nitrification inhibitor manufactured by Ishihari Industries, Japan. The treatments were replicated four times. Ten millilitres of N solution (180 mg N·cylinder^{-1}) equivalent to 56 kg N·ha^{-1} were injected into the soil at a depth of 10 cm. ATC was used at a rate of 4% of fertilizer N application rate (wt/wt basis). The ^{15}N excess of each carrier was 5.6%. The fertilizers were applied in fall 1976 and spring 1977. Phosphate fertilizer was added at seeding time (20 kg P·ha^{-1}). The wheat was harvested at maturity, cylinders were removed from the soil and soil cores (10 cm in diameter and 30 cm deep) were taken from the soil beneath the cylinder. In the laboratory, the cylinders were cut open and the soil was sectioned into A (0–15 cm), AB (15–30 cm) and B (30–60 cm) layers and air-dried. Plant and soil subsamples obtained from the microplots were ground to pass a 100-mesh sieve and analyzed for total N, including NO_3^--N and NO_2^--N, using a modified version of the semi-micro method of Bremner (1965a). Soil samples were pretreated with acidified permanganate to oxidize NO_2^--N to NO_3^--N, and with reduced iron and sulfuric acid to reduce NO_3^--N to NH_4^+-N.

Incubation Experiments

An incubation experiment was carried out using field ^{15}N enriched surface (0–15 cm) soil samples taken from NH_4OH, NH_4OH + ATC and urea + ATC-treated plots. The soils were preincubated for 3 wk at 28 ± 1°C and 34 kPa soil pore water potential and sampled at 0, 2, 4, 6, 9 and 12 wk. Nonexchangeable NH_4^+, biomass N and their ^{15}N abundances were determined.

Analyses

The technique for nonexchangeable NH_4^+ in the surface soil samples (Bremner 1965b) involved treatment of 1 g soil (<100 mesh) with 20 mL of freshly prepared alkaline potassium hypobromite (KOBr-KOH) solution to oxidize organic N, washing the residue with 0.5 mol KCl to remove exchangeable NH_4^+ and treating the soil with 5 mol HF-1 mol HCl to decompose minerals containing nonexchangeable NH_4^+. The NH_4^+ released by the HF-HCl treatment was determined by steam distillation after the addition of KOH to the soil-acid mixture. Isotope ratio analysis was performed on an Atlas GD150 or a Micromass 602E mass spectrometer.

A freeze-dried sample of NH_4OH + ATC-treated soil was fractionated into various particle sizes. The method involved dispersing the soil by shaking one part of soil in five parts H_2O for 15 min. The sample was sonicated for 2 min at 300 W. Sand-size particles were separated by sieving (300 mesh) and silt- and clay-size particles by centrifugation (Jackson 1975).

The soil particle size fractions were prepared for X-ray diffraction analysis (Jackson 1975) as follows: A 50-mg sample was treated with 1 mol sodium acetate (pH 5) to dissolve free carbonates and with H_2O_2 to destroy organic matter. The sample was Mg-saturated using 2 mol $MgCl_2$, washed free of excess chlorides, glycerol solvated and dried in a desiccator. Another 50-mg sample was taken through K saturation (Jackson 1975). X-ray diffraction analysis was done with a Phillips diffractometer with Cu target element and a Ni filter. Settings of 35 kV

and 16 mA were used to generate X-rays and the 2θ angle was set at 3°. A Geiger-counter detector with a chart recorder was used. The K-saturated sample was heated at 110, 300 and 500°C and X-rayed each time after cooling in a desiccator. The nonexchangeable NH_4^+ and ^{15}N abundance of each particle size fraction were also determined.

Duplicate, 50-g moist soil samples (ODB) were used for biomass N determination (Jenkinson and Powlson 1976) as follows: the samples were exposed to ethanol-free chloroform vapors for 24 h in a desiccator, followed by evacuation of these vapors and subsequent incubation for 10 days at 28 ± 1°C. Thus,

$$\text{Biomass N} = \frac{NH_4^+ \text{ accumulated during 10 days } (\mu g \cdot g^{-1} \text{ soil})}{k_N}$$

where k_N, the conversion factor, was equal to 0.30 (Voroney and Paul 1983.)

RESULTS

Disposition of Fertilizer N

There were no significant differences ($P<0.05$) in the recovery or utilization of ^{15}N aqua NH_3 or urea with or without ATC in the grain, straw and soil due to the time of application (data not shown). Therefore, the data for the fall and spring applications were pooled. There were no significant differences ($P<0.05$) in the utilization of ^{15}N aqua NH_3 or urea with or without ATC in the grain and straw due to the use of nitrification inhibitor (Table 1). The recovery of ^{15}N in the grain ranged from 24 to 28% while the recovery in the straw ranged from 10 to 12% for a total recovery of 35–39% fertilizer N in the aboveground plant parts.

The ^{15}N in soil included both soil and roots. The amount of fertilizer N found in the 0- to 15-cm layer (52–55%) in the ATC treatments was significantly greater ($P<0.01$) than that found in the non-ATC treatments (29–31%; Table 1). The converse was true in the 15- to 30-cm layer ($P<0.05$). The total amount of fertilizer N recovered in the soil profile was significantly greater in the ATC plots (55–60%) compared to the non-ATC plots (40–42%). The larger quantities of fertilizer N found in the 15- to 30-cm layers in the non-ATC treatments could be explained by nitrification of fertilizer N and its subsequent leaching or downward movement. Only a small quantity of fertilizer N (<2%) was found below 30 cm. Total recovery of fertilizer N in the soil-plant system was generally greater in the ATC treatments (90–95%) compared to the non-ATC treatments (80–82%).

Disposition of Nonexchangeable $^{15}NH_4^+$

Nonexchangeable NH_4^- ions are absorbed or adsorbed by mineral fractions of the soil in such a way that the ions are relatively water insoluble and unexchangeable by the

Table 1. Recovery or utilization of ^{15}N-labeled solution ammonia and urea with and without ATC in grain, straw and soil

	Treatments			
Recovery in	NH_4OH + ATC	NH_4OH	Urea + ATC	Urea
	% recovery or utilization†			
Grain	24 *c*	28 *c*	25 *c*	27 *c*
Straw	11 *c*	11 *c*	11 *c*	12 *c*
0–15 cm	56 *a*	29 *b*	52 *a*	31 *b*
15–30 cm	3 *c*	10 *d*	2 *c*	10 *d*
30–60 cm	1 *c*	2 *c*	1 *c*	2 *c*
Total soil	60 *c*	40 *d*	55 *c*	43 *d*
Total	95 *c*	79 *d*	91 *cd*	82 *d*

†Group means were compared using the Scheffe test.
a,b,c,d Group means in each horizontal category followed by the same letters are not significantly different at $P < 0.01$ (*a* and *b*) and $P < 0.05$ (*c* and *d*).

usual methods of cation exchange (Agriculture Canada 1976). There was no significant difference in the total nonexchangeable NH_4^+ ($P<0.05$) of the various treatments with an average of 128 μg NH_4^+-N·g^{-1} soil. However, 5–8% of fertilizer N was recovered in the nonexchangeable NH_4^+ fraction of the ATC treatments compared to 1% in the non-ATC treatments ($P<0.01$). There was no significant difference due to the fertilizer carrier used or due to time of application.

The concentration of nonexchangeable NH_4^+ ranged from 41 μg N·g^{-1} sand to 318 μg N·g^{-1} clay (Table 2). The coarse clay and coarse silt fractions accounted for 70% of total nonexchangeable NH_4^+. About 22% of the total nonexchangeable N was equally distributed in the fine silt and fine clay fractions. The sand fractions accounted for 8% of the total nonexchangeable NH_4^+.

About one-half of the ^{15}N in the nonexchangeable fraction was found in the coarse clay and about one-fourth in the coarse silt fraction. Smaller amounts of ^{15}N were found in the fine silt and fine clay fractions; with none in the sand fraction. This indicates that the coarse clay fraction which accounted for 37% of the total exchangeable NH_4^+ was most active in the fixation of $^{15}NH_4^+$.

Quartz was present in all the particle size fractions (Table 3). K-feldspars and plagioclase feldspars were present in the sand, coarse silt and fine silt fractions. It is possible that these could contain some geological nonexchangeable NH_4^+. The coarse silt fraction contained mica which is known to fix NH_4^+. The presence of mica, vermiculite and smectites in the fine silt and coarse clay was concomitant with high nonexchangeable NH_4^+ concentrations in these fractions (Tables 2 and 3).

The activity of each of the particle size fractions depends upon the proportion of soil weight present in the particle size fraction and the minerals present in the fraction. The coarse silt fraction accounted for 40% of the total weight of soil; it contained mica and feldspars and retained 26% of the total $^{15}NH_4^+$. The coarse clay accounting for 16% of the soil weight, but containing mica, smectites and vermiculite, retained 49% of the total labeled nonexchangeable NH_4^+. These results indicate that vermiculite was the major mineral involved in the fixation of NH_4^+. The absence of vermiculite in the fine clay fraction resulted in a lower proportion of fixed labeled NH_4^+.

Table 2. Disposition of ^{15}N in the nonexchangeable NH_4^+ in various particle size fractions of Weirdale loam soil treated with $^{15}NH_4OH$ + ATC†

Fraction	% of total wt	Nonexchangeable NH_4^+-N			Nonexchangeable $^{15}NH_4^+$-N		
		μg·g^{-1} fraction	μg·g^{-1} soil	% of total	Atom % ^{15}N abundance	^{15}N remaining (ng·g^{-1} soil)‡	% of total labeled
Sand	26	41	11	8	0.3633	0	0
Coarse silt	40	111	44	33	0.3990	13	26
Fine silt	6	229	13	10	0.4235	7	14
Coarse clay	16	317	49	37	0.4184	24	49
Fine clay	5	318	16	12	0.4035	6	11
Organic matter	7	–	–	–	–	–	–
Total	100		133			50	
Whole soil	–	–	133	–	0.4066	50§	–

†The atom percent ^{15}N abundance of nonexchangeable NH_4^+ was 0.3697 in the untreated soil.
‡^{15}N remaining = total nonexchangeable NH_4^+ (μg·g^{-1}) × (atom percent ^{15}N excess/100) × 1000 ng/μg.
§This was equivalent to 2.8 kg N·ha^{-1} (5% of the applied fertilizer N).

Table 3. Mineralogy of different particle size fractions of Weirdale loam soil

Fraction and size	Mineral†								
	Quartz	K-feldspars	Plagioclase feldspars	Mica	Kaolinite	Smectites	Amphiboles	Vermiculite
Sand 50 μm	+	+	+					
Coarse silt 50-5 μm	+	+	+	+	+		+	
Fine silt 5-2 μm	+	+	+	+	+	+		+
Coarse clay 2-0.2 μm	+			+	+	+		+
Fine clay 0.2 μm	+			+	+	+		

†Mineral was present and identified by X-ray diffraction techniques.

Rate of Release of Nonexchangeable $^{15}NH_4^+$

The release of nonexchangeable $^{15}NH_4^+$-N on incubation of the ATC-treated urea or aqua ammonia soil could be described by first order kinetics. The half-lives of nonexchangeable ammonium in these soils ranged from 35 to 42 wk (Fig. 1). The NH_4OH-treated soil was also incubated and showed a declining trend. However, it was difficult to establish any significant relationship due to the small amount of ^{15}N in this fraction (5 $ng \cdot g^{-1}$). A total of 5% of the remaining ^{15}N was present as nonexchangeable NH_4^+ in the NH_4OH + ATC-treated soil. The nonexchangeable $^{15}NH_4^+$ accounted for 8% of the ^{15}N remaining in the soil after fertilization with urea + ATC. The urea + ATC-treated soil contained more ^{15}N and had a faster rate of release than the NH_4OH + ATC-treated soil. Present techniques do not allow separation of nonexchangeable NH_4^+ into peripheral interlayer, interlayer and interstitial nonexchangeable NH_4^+.

Disposition of Fertilizer in Microbial Biomass and Rate of ^{15}N Release from Microbial Biomass

The biomass ^{15}N, calculated using $CHCl_3$ fumigation and a k_N of 0.30, accounted for 36–50% of the soil organic ^{15}N remaining (Table 4). Biomass ^{15}N in the NH_4OH and urea + ATC soil had half-lives ranging from 24.7 to 27.2 wk (Table 4). The biomass ^{15}N in the NH_4OH + ATC soil had a slightly longer half-life (33.9 wk). The net decay rate of biomass ^{15}N was also calculated by expressing all the data on a relative basis. The initial pool sizes were set to 100%. The regression equation describing the decay of biomass ^{15}N in all these soils was:

$$\% \text{ biomass remaining} = 99.3e^{-0.025t}$$
$$r^2 = 0.90^{**}$$
$$t\ 1/2 = 27.6 \text{ wk}$$

The relative regressed pool size was 99% of the initial value (100%) with an average half-life of 27.6 wk.

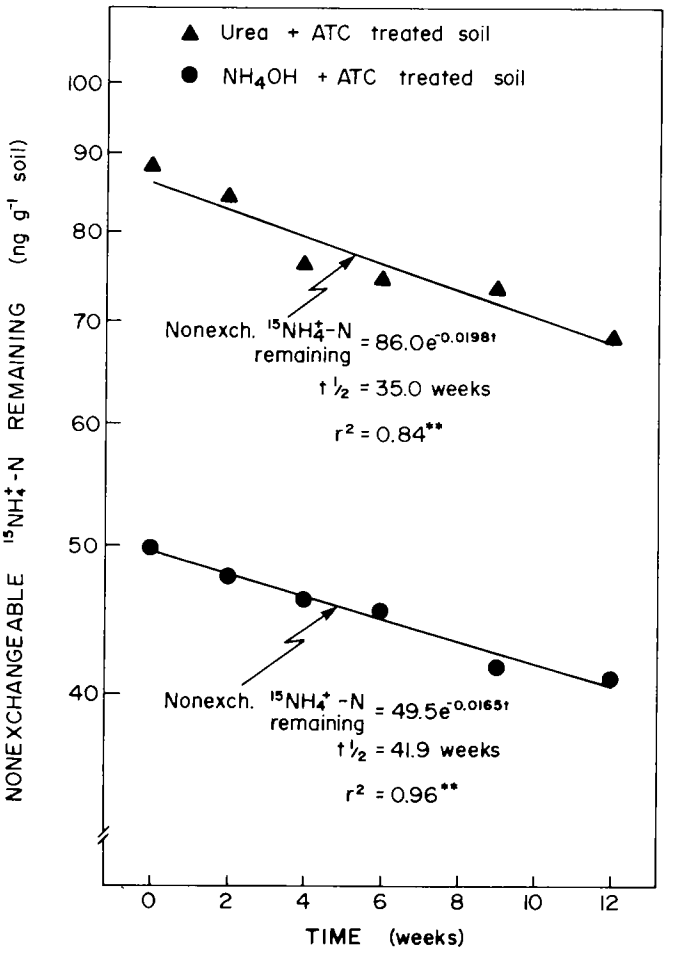

Fig. 1. Release of nonexchangeable $^{15}NH_4^+$-N as a function of time.

DISCUSSION

Nitrification inhibitors can increase the recovery of fertilizer N by delaying the nitrification of ammoniacal fertilizers, thus lowering subsequent losses by leaching or denitrification. ATC markedly inhibited the movement of fertilizer N from the surface to subsurface horizons and increased the amount remaining in the soil although plant uptake during one growing season was not affected. It increased the recovery of fertilizer N by enhancing biological immobilization and conversion of ammoniacal N into nonexchangeable N. The immobilized fertilizer N becomes available over a number of years but its immediate benefit to plant growth is low.

The dynamics of nonexchangeable NH_4^+ in soil are governed by a number of factors such as the size, amounts and kinds of minerals present, and the presence of K^+ and NH_4^+. The average net half-life of nonexchangeable $^{15}NH_4^+$-N for the surface Weirdale loam soil treated with aqua NH_3 + ATC or solution urea + ATC was 38.5 wk at 34 kPa and 28 ± 1°C. The half-life of nonexchangeable NH_4^+ in the aqua NH_3-treated soil was not determined be-

Table 4. Net decay of microbial biomass ^{15}N in Weirdale loam soil during a 12-wk laboratory incubation

Treatment	$^{15}NH_4^+$ produced on fumigation/ incubation ($ng \cdot g^{-1}$ soil)	^{15}N in biomass ($ng\ ^{15}N \cdot g^{-1}$ soil)+	% of total organic ^{15}N remaining†	Decay rate constant (mean ± SE)	r^2	$t_{1/2}$ (week)
NH_4OH-1‡	18	60	41	0.028 ± 0.003	0.98**	24.7
NH_4OH-2‡	14	47	36	0.026 ± 0.001	0.99**	27.2
NH_4OH + ATC	38	127	42	0.020 ± 0.003	0.92**	33.9
Urea + ATC	35	117	50	0.026 ± 0.004	0.96**	26.2

†On the basis that biomass = NH_4^+/0.30.
‡Similar treatments which were incubated at separate times.
**Denotes statistical difference at $P < 0.01$.

cause it contained a very small amount of fertilizer N. The studies showed that the coarse clay accounted for 37% of the total nonexchangeable NH_4^+ but contained 49% of the total nonexchangeable $^{15}NH_4^+$-N, whereas the coarse silt fraction accounting for 33% of nonexchangeable NH_4^+ had 26% of the $^{15}NH_4^+$-N. These particle size fractions had mica and vermiculite, which have high fixation capacities, and smectites which have moderate fixation capacities. A variety of factors affect the release of N from the nonexchangeable NH_4^+ fraction and the data reported here indicate that nonexchangeable NH_4^+ is not an inert N fraction in the soil although ^{15}N was only incorporated into this fraction in the presence of ATC.

The bulk of fertilizer N remaining at the end of the season was present in organic forms. There was almost twice as much $^{15}NH_4^+$ extracted after fumigation/incubation of the ATC-treated soils compared to the non-ATC-treated soil. The net release of this ^{15}N in the laboratory incubation was faster ($t_{1/2}$ = 27.6 wk) compared to the nonexchangeable $^{15}NH_4$ ($t_{1/2}$ = 38.5 wk) and quantitatively more ^{15}N would be released from the microbial biomass due to its large pool size. Therefore, the biologically immobilized fertilizer would gradually be released over a number of years. This would result in a gradual decline of the atom percent ^{15}N abundance of NO_3^--N during incubation (Broadbent and Nakashima 1967) or of total N of several subsequent crops (Legg et al. 1971).

The problems involved in converting values for the NH_4^+ released on fumigation/incubation to biomass N have been discussed by Voroney and Paul (1983). On the basis of isotopic dilution calculations, they suggested a conversion factor (k_N) of 0.30 for soils where most of the population represents long-term steady state conditions with adequate available N. The ^{15}N present in the biomass in this study represented the microbial population after a season's growth in the field in the presence of fertilizer N; thus, the k_N suggested by Voroney and Paul should be applicable. Ladd

et al. (1981) assumed a k_N that was twice that reported by Voroney and Paul. This indicates that although the $CHCl_3$-fumigation/incubation technique is exceptionally useful in estimating ^{15}N mineralization/immobilization rates, the actual biomass N involved is still open to question.

The net half life of microbial biomass ^{15}N is an average because there are a variety of microbial populations with different half-lives. Also, the decaying biomass contains several N components such as DNA, RNA, proteins and peptidoglycan which may have different decomposition rates. However, the biomass as a unit is an active organic N pool and would release the ^{15}N at a more rapid rate than the recalcitrant and stable organic materials in soils.

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