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Fate of Nitrogen-15 Enriched Ammonium Nitrate Applied to Corn

G. B. Reddy* and K. R. Reddy

ABSTRACT

Nitrogen utilization by corn (*Zea mays* L.) is influenced by the form of inorganic N present in the root zone. A field experiment was conducted on Enon sandy loam (fine, mixed, thermic Ultic Hapludalf) to determine N use efficiency and its partitioning in various plant parts of corn. Ammonium nitrate labeled either as $^{15}NH_4$–N or $^{15}NO_3$–N and applied at 50, 100, or 200 kg N ha^{-1} was evaluated. Microplots (1.06 m^2) were established in the main N plots for ^{15}N fertilizer application, and main plots were used to determine grain yield. After the crop harvest, soil N alone, fertilizer N used by the crop, and fertilizer N remaining in inorganic and organic forms in the top 75 cm of the soil were measured. Grain yield response to N application was significant ($P < 0.01$). Recovery of applied fertilizer N in corn and weeds ranged from 43 to 57% and 3 to 5%, respectively. Nitrogen sources showed no significant differences with respect to N accumulation in corn. Only 17 to 20% of the fertilizer N was recovered in the grain. The amounts of soil N utilized by the corn was about three- to sixfold higher than the fertilizer N, suggesting extensive turnover of soil and fertilizer N through immobilization and mineralization. More N was recovered (21–63% of added N) in the soil from $^{15}NH_4$ than from $^{15}NO_3$ (6–38%). Loss of N (unaccounted for) ranged from 11 to 18% at 100 kg N ha^{-1} and from 34 to 48% at 200 kg N ha^{-1}. Nitrogen loss was higher in the plots receiving $^{15}NO_3$ than $^{15}NH_4$. Most of the fertilizer N remaining in the soil at the end of the growing season was in the organic fraction, suggesting immobilization into microbial and root biomass.

NITROGEN USE EFFICIENCY by corn is influenced by source, rate and time of fertilizer application, and hydrologic conditions such as rainfall distribution and water-table fluctuations during the growing season. The use of fertilizer labeled with the stable isotope ^{15}N provides a meaningful way to differentiate between the total amount of fertilizer N utilized by the crop and N losses during crop production for both environmental and agronomic reasons. Many researchers (e.g., Carter et al., 1967; Westerman et al., 1972; Bijeriego et al., 1979; Olson, 1980) have studied the recovery of labeled fertilizers in corn and sudangrass [*Sorghum* × *drummondii* (Steudel) Millsp. & Chase] at the time of harvest. Kitur et al. (1984) reported that 71 to 75% of the fertilizer N was accounted for in harvested grain and stover of corn and soil. Nitrogen rate had no large effect on the fraction of fertilizer N unaccounted for and presumed to be lost by denitrification and leaching. Chabrol et al. (1988) found that, when a mixture of $^{15}(NH_4)_2SO_4$ and $K^{15}NO_3$ (122 kg N ha^{-1}) was applied to corn 12 d after planting, 61% was present in the crop and 19% remained in the soil; the 20% loss observed was assumed to be due to a combination of leaching and denitrification. Hera (1977) observed that, when labeled NH_4NO_3 was applied at the rate of 160 kg N ha^{-1} in split application, 47 and 14 kg N ha^{-1} were recovered in grain and stalk, respectively, and the efficiency of fertilizer utilization was 38%. Sanchez and Blackmer (1988) found that 13 to 33% of the labeled N was recovered in corn grain and 19 to 23% in soil after application of ^{15}N-labeled anhydrous NH_3.

Inorganic N occurs in most soils as NH_4^+ and NO_3^-, and in upland soils NO_3^- is the predominant form of inorganic N available to plants. Therefore, it is important to know which form of inorganic N is highly available to corn and its partitioning in the soil–plant system. The studies reported above present very little information on the partitioning of applied fertilizer ^{15}N in plant parts of corn at the end of the growing season. This information may provide insight on N use efficiency and translocation, especially for N-limited systems.

The objectives of this study were to (i) quantify the fertilizer ^{15}N uptake by corn, and its partitioning in various plant parts; (ii) determine the N use efficiency by corn; and (iii) estimate fertilizer N loss using mass balance.

MATERIALS AND METHODS

The field experiment was conducted in 1986 on an Enon sandy loam soil, at the North Carolina A&T State University farm in Guilford County, North Carolina. The surface soil characteristics were: pH, 6.0; CEC, 7.0 cmol$_c$ kg^{-1}; soil total N, 0.31 g kg^{-1}; total inorganic N, 21 mg kg^{-1}; and organic matter content, 8.1 g kg^{-1}.

The field plots were tilled before planting. The plot size was 4.57 by 7.62 m and was planted with six rows of corn ≈0.91 m apart. Two weeks prior to planting, 3.51 L ha^{-1} butylate (S-ethyl diisobutylthiocarbamate) and 2.35 L ha^{-1} atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) were mixed in an appropriate volume of water and applied to the plots. Pioneer 3369AR corn was planted on 8 May 1986, at a rate to obtain 43 078 plants ha^{-1}. Ammonium nitrate was applied at the rate of 0, 50, 100, or 200 kg N ha^{-1}. Recommended rates of P (60 kg ha^{-1}) and K (60 kg ha^{-1}) were applied to all the plots. Planting and fertilizer application were done by hand. Fertilizers were applied in bands ≈5 cm deep and 8 cm away from the center of each row. Treatments were replicated three times in a randomized complete-block experimental design. Average ambient minimum and maximum air temperatures during the growing season were 16 and 27 °C, respectively. The total precipitation during the growing season was 170 mm.

For determination of fertilizer N recovery, ^{15}N-enriched fertilizer was applied to microplots (1.16 by 0.92 m) within each large plot. Two microplots were established permanently in the center of each main plot by inserting a galvanized metal (1-mm-thick) barrier into the soil to a depth of 0.31 m. Two microplots in each main plot were 2.14 m apart. One microplot was treated with $^{15}NH_4NO_3$ (10.387 atom %) and another with $NH_4{}^{15}NO_3$ (10.396 atom %), at respective N levels of each treatment. Enriched ^{15}N fertilizer was placed in bands as described for the main plot. Due to the dry season, plots were irrigated (50 mm ha^{-1}) once in May and twice in June. Plots were hand weeded and weed plants were washed, dried, and saved for chemical analysis.

At harvest, plants in the center rows were harvested for

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Abbreviations: CEC, cation-exchange capacity; Ndff, plant N derived from labeled fertilizer N; Ndfs, soil N plus nonlabeled fertilizer N; Nds, native soil N.

Table 1. Plant N derived from labeled fertilizer N (Ndff), soil N plus nonlabeled fertilizer N (Ndfs), and native soil N (Nds). Fertilizer applied as NH_4NO_3 at planting.

Plant part	N rate	$^{15}NH_4NO_3$ Total N	Ndf_{15NH_4}	Ndfs	Nds	$NH_4{}^{15}NO_3$ Total N	Ndf_{15NO_3}	Ndfs	Nds
		kg N ha⁻¹							
Leaves	50	24.4	3.2	21.3	17.7	20.9	3.6	17.4	14.2
	100	29.2	6.3	22.9	14.8	35.0	8.1	23.5	17.2
	200	46.4	12.8	33.6	21.7	44.7	11.7	33.0	20.2
Stem	50	19.5	3.4	16.1	13.6	16.6	2.5	14.0	10.6
	100	23.2	5.8	17.4	11.4	30.5	6.0	24.5	18.7
	200	37.9	6.1	31.8	23.2	38.1	8.6	29.4	23.3
Ear leaf	50	5.7	0.9	4.9	4.2	4.5	0.7	3.8	2.9
	100	10.2	2.0	8.3	6.4	8.6	1.9	6.7	4.7
	200	12.0	2.8	9.2	7.4	7.1	1.8	5.3	2.5
Tassel	50	1.6	0.3	1.3	0.9	1.9	0.4	1.4	1.1
	100	2.3	0.5	1.9	1.3	2.5	0.6	1.9	1.4
	200	1.9	0.4	1.5	0.8	2.2	0.7	1.6	1.2
Cob	50	9.2	1.9	7.2	5.9	8.9	1.3	7.0	5.1
	100	13.6	2.7	10.8	8.1	13.0	2.7	10.3	7.6
	200	14.5	3.6	10.9	7.8	10.0	3.1	6.8	3.2
Seeds	50	24.5	4.5	20.0	15.1	32.3	4.9	27.5	23.0
	100	29.0	5.9	23.0	14.0	56.5	9.0	47.5	41.6
	200	89.2	17.1	72.1	54.4	55.3	17.6	37.7	20.6
Weeds	50	8.6	0.8	7.9	6.8	14.4	1.1	13.3	12.5
	100	7.7	0.9	6.8	6.3	12.8	0.5	12.4	11.5
	200	14.4	2.2	12.2	10.0	17.1	2.2	14.9	12.7

Table 2. Statistical data (for the results presented in Table 1) showing the significant effects of ^{15}N fertilizer (Ndff) and soil plus nonlabeled fertilizer N (Ndfs) assimilation in various plant parts.

Plant part	Treatment	Total N	Ndff	Ndfs
		$P > F$		
Leaves	N rate	0.01	0.01	0.01
	N source	NS†	NS	NS
	N rate × N source	NS	NS	NS
Stem	N rate	0.01	0.03	0.01
	N source	NS	NS	NS
	N rate × N source	NS	NS	NS
Ear leaf	N rate	0.03	0.02	0.05
	N source	0.07	NS	NS
	N rate × N source	NS	NS	NS
Tassel	N rate	NS	NS	NS
	N source	NS	0.03	NS
	N rate × N source	NS	NS	NS
Cob	N rate	0.01	0.01	0.02
	N source	0.09	NS	NS
	N rate × N source	NS	NS	NS
Seeds	N rate	0.03	0.02	0.06
	N source	NS	NS	NS
	N rate × N source	NS	NS	NS
Weeds	N rate	NS	0.04	NS
	N source	NS	NS	0.09
	N rate × N source	NS	NS	NS

† NS indicates a probability >0.10.

yield. Plants in the microplots were harvested and separated into different plant portions (leaf, stem, cob, ear-leaf, and grain). All plant portions were dried at 70 °C, and weights were recorded. The plant tissue was ground and analyzed for total N using the Kjeldahl digestion method (Bremner and Mulvaney, 1982) and for labeled N (Hauck, 1982). Soil samples were collected at five depths (0–15, 15–30, 30–45, 45–60, and 60–75 cm) from four locations in each microplot and composited. Soils were air dried, crushed, and sieved through a 2-mm screen. A known amount of soil was extracted with 2 *M* KCl (1:10 ratio) after 1 h of shaking, and filtered solutions were acidified to pH 2 and frozen until analyzed. The residual soil retained on the filter paper was washed with distilled water, followed by drying at 60 °C. The filtered solutions were analyzed for NH_4–N and NO_3–N using steam distillation (Keeney and Nelson, 1982), while the residual soil was analyzed for total N (Bremner and Mulvaney, 1982). All N fractions were subsequently analyzed for labeled N content (Hauck, 1982). The labeled N (^{15}N) content of the samples was analyzed using a Micromass 602E isotope ratio mass spectrometer (VG Instruments, West Sussex, England).

Calculations

Fertilizer N use by corn from two ^{15}N sources, $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$, was evaluated. Thus, in each field plot, corn derived N from three sources: (i) labeled fertilizer N, (ii) nonlabeled fertilizer and soil N, and (iii) soil N alone. The contribution of each source to plant N was calculated as follows.

Microplots receiving $^{15}NH_4NO_3$:

$$Ndf_{15NH_4} = TN\left[\frac{(A - B)}{(C - B)}\right] \quad [1]$$

where TN = total N uptake by the plant; A = atom % ^{15}N in the plant tissue; B = atom % ^{15}N in the natural standard; C = atom % ^{15}N in the $^{15}NH_4NO_3$ fertilizer applied; and Ndf_{15NH_4} = fertilizer ^{15}N uptake from $^{15}NH_4NO_3$ source.

Microplots receiving $NH_4{}^{15}NO_3$:

$$Ndf_{15NO_3} = TN\left[\frac{(A - B)}{(D - B)}\right] \quad [2]$$

where D = atom % ^{15}N in the $NH_4{}^{15}NO_3$ fertilizer added and Ndf_{15NO_3} = fertilizer ^{15}N uptake from $NH_4{}^{15}NO_3$ source.

Nitrogen derived from soil N alone was calculated as follows with an assumption that plants did not discriminate between ^{15}N and ^{14}N sources of fertilizer N:

$$Nds = TN - (Ndf_{15NH_4} + Ndf_{15NO_3}) \quad [3]$$

Table 3. Distribution of fertilizer ^{15}N in the soil by depth at the end of the corn growing season as influenced by fertilizer N source and rate.

	Fertilizer ^{15}N content					
	0–15 cm	15–30 cm	30–45 cm	45–60 cm	60–75 cm	Total
	kg N ha^{-1}					
			50 kg N ha^{-1}			
$^{15}NH_4NO_3$						
NH_4–N	0.20	0.05	0.07	0.07	0.13	0.52
NO_3–N	0.33	0.04	0.08	0.08	0.13	0.66
Total inorganic N	0.53	0.09	0.15	0.15	0.26	1.18
Organic N	5.77	2.23	1.60	2.22	2.65	14.47
Total N	6.30	2.32	1.75	2.37	2.91	15.65
$NH_4{}^{15}NO_3$						
NH_4–N	0.06	0.02	0.04	0.03	0.02	0.17
NO_3–N	0.02	0.00	0.01	0.01	<0.01	0.04
Total inorganic N	0.08	0.02	0.05	0.04	0.02	0.21
Organic N	5.02	1.12	0.51	1.96	0.73	9.34
Total N	5.10	1.14	0.56	2.00	0.75	9.55
			100 kg N ha^{-1}			
$^{15}NH_4NO_3$						
NH_4–N	0.17	0.05	0.06	0.09	0.27	0.64
NO_3–N	0.18	0.03	0.05	0.01	0.00	0.27
Total inorganic N	0.35	0.08	0.11	0.10	0.27	0.91
Organic N	8.96	2.24	2.38	2.64	3.22	19.44
Total N	9.31	2.32	2.49	2.74	3.49	20.35
$NH_4{}^{15}NO_3$						
NH_4–N	0.50	0.16	0.06	0.66	0.22	1.60
NO_3–N	0.17	0.07	0.03	0.05	0.02	0.34
Total inorganic N	0.67	0.23	0.09	0.71	0.24	1.94
Organic N	4.48	2.33	1.42	0.93	1.13	10.29
Total N	5.15	2.56	1.51	1.64	1.37	12.23
			200 kg N ha^{-1}			
$^{15}NH_4NO_3$						
NH_4–N	0.33	0.12	0.26	0.21	0.09	1.01
NO_3–N	1.17	0.19	0.19	0.12	0.08	1.75
Total inorganic N	1.50	0.31	0.45	0.33	0.17	2.76
Organic N	9.55	2.67	3.44	1.42	1.56	18.64
Total N	11.05	2.98	3.89	1.75	1.73	21.40
$NH_4{}^{15}NO_3$						
NH_4–N	0.05	0.03	0.04	0.001	0.22	0.34
NO_3–N	0.10	0.07	0.08	0.00	0.08	0.33
Total inorganic N	0.15	0.10	0.12	0.001	0.30	0.67
Organic N	1.03	2.40	0.84	0.00	1.09	5.36
Total N	1.18	2.50	0.96	0.00	1.39	6.03

where Nds = N derived from soil N alone.

Nitrogen derived from nonlabeled fertilizer and soil N was calculated as follows:

$$\text{Ndfs} = \text{TN} - \text{Ndf}_{^{15}\text{NH}_4} \quad [4]$$

or

$$\text{TN} - \text{Ndf}_{^{15}\text{NO}_3} \quad [5]$$

where Ndfs = N derived from nonlabeled fertilizer N and soil N.

RESULTS AND DISCUSSION

Grain Yield

A significant response ($P < 0.01$) was observed between N rate and grain yield. The recorded grain yield was 3.15, 3.90, 4.55, and 5.51 Mg ha^{-1} at 0, 50, 100, and 200 kg N ha^{-1}, respectively. Generally, grain yield was lower than that observed typically for the Piedmont region, because fertilization and planting were delayed due to the long period of drought and high temperatures in the months of June and July at the time of initiation of silking. Source of N showed no effects on grain yield.

Distribution of Nitrogen in Plant Parts

Table 1 shows the partitioning of Ndff, Ndfs, and Nds in various plant parts of corn, as influenced by N rate and source. Nitrogen rate significantly influenced both Ndff and Ndfs accumulation in leaves, stems, ear-leaf, cob, and grain (P = 0.01–0.06) (Table 2). Fertilizer N and Ndfs were highest in the seeds followed by leaves, stems, cob, ear-leaf, and tassel. Grain accumulated 25 to 39 and 32 to 41% of fertilizer N accumulated in plants at 50 and 200 kg N ha^{-1}, respectively. Ear-leaf, tassel, and cob together accumulated 22, 18, and 14% of fertilizer N, when corn was grown at 50, 100, and 200 kg N ha^{-1}, respectively. Similar N recoveries have been reported by several researchers: 35% (Bijeriego et al., 1979), 14 to 65% (Meisinger et al., 1985), 23 to 45% (Kitur et al., 1984), 24 to 26% (Olson, 1980), and 15 to 33% (Sanchez and Blackmer, 1988). There was no significant difference with respect to N accumulation in corn (Table 2) with either NO_3^- or NH_4^+ forms of N. Similarly, Schrader et al. (1972), and Warnke and Barber (1973) found no significant differences between NH_4^+ and NO_3^- absorption by corn. In upland soils, NH_4^+ is rapidly converted to NO_3^-, resulting in the availability of NO_3^- to the plant during the major part of the growing season. Use of nitrification inhibitors could have reduced this conversion, but these inhibitors are not always effective (Harris, 1990). Accumulation of fertilizer N in weeds also increased with N rate, with maximum accumulation of 2.2 kg N ha^{-1} at 200 kg N ha^{-1} application.

Plant accumulation of N from Nds increased with fertilizer N application. The apparent increase in the uptake of soil N with increased N rates could be due to increased mineralization from soil organic matter or due to pool substitution between ^{14}N and ^{15}N in the soil (Jenkinson et al., 1985; Hart et al., 1986; Rao et al., 1991).

Distribution of Fertilizer Nitrogen in Soil

Distribution of fertilizer N in the soil profile as influenced by N rate and source is presented in Tables 3 and 4. The rate of N fertilizer, N source, and soil depth showed significant ($P < 0.01$) effects on fertilizer NO_3–N, organic N, and total N in the soil (Table 4). Fertilizer N recovered either as NH_4^+ or NO_3^- showed significant differences with depth ($P < 0.01$). Fertilizer $^{15}NH_4$–N recovery in the soil was higher than the recovery of fertilizer $^{15}NO_3$–N. Both $^{15}NH_4$–N and $^{15}NO_3$–N were recovered even at depths of 60 to 75 cm, suggesting leaching of NO_3^- below the root zone. Below 15 cm, however, the amounts of mineral forms (NH_4^+ and NO_3^-) available in the soil were negligible. Labeled N recovery in the organic N fraction was higher at the surface and decreased with depth. Approximately one-half of the residual N was recovered from the surface 0 to 15 cm. Most of the residual N was immobilized in the organic fraction and was relatively stable, resulting in minimal downward movement of inorganic ions. Studies reported in the literature have also concluded that most of the fertilizer N that remained in the soil was bound in organic form after the first cropping season (Legg et al., 1971; Allen et al., 1973; Olson, 1980; Power and Legg, 1984).

Table 4. Statistical data for the results (for the data presented in Table 3) showing fertilizer NH_4–N and NO_3–N in the soil, as influenced by fertilizer N source and rate.

Treatment	NH_4–N	NO_3–N	Total inorganic N	Organic N	Total N
			$P > F$		
N rate	0.01	0.01	0.01	0.03	0.03
N source	NS†	0.01	0.02	0.01	0.01
Soil depth	0.01	0.01	0.01	0.01	0.01
Rate × source	0.01	0.01	0.01	0.01	0.01
Rate × depth	NS	0.01	NS	0.06	0.10
Source × depth	NS	0.01	0.01	0.01	0.01

† NS indicates probability level >0.10.

Mass Balance of Fertilizer Nitrogen

Fertilizer ^{15}N recovery from $^{15}NH_4$ and $^{15}NO_3$ sources in soil and plant components is shown in Table 5. After one growing season, recovery of N from $^{15}NH_4$ and $^{15}NO_3$ fertilizer by the corn was in the range of 43 to 56 and 44 to 57% of added N, respectively. These crop uptake values for labeled fertilizer N were similar to the findings of other researchers (Krauter, 1975; Broadbent and Krauter, 1974; Bijeriego et al., 1979; Chabrol et al., 1988). Less than 3% of added $^{15}NH_4$ and 5% of added $^{15}NO_3$ were recovered in weeds. More N was recovered in the soil from the $^{15}NH_4$ source (21–63% of added N), compared with recovery from the $^{15}NO_3$ source (6–38% of added N). Nitrogen loss (N unaccounted for) from the soil–plant system was higher from $^{15}NO_3$ sources than $^{15}NH_4$ sources. At the highest rate of fertilizer N (200 kg N ha^{-1}), 34% of the added NH_4–N was unaccounted for, compared with 48% of the added NO_3–N. At 100 kg N ha^{-1}, the N loss ranged from 11 to 18%. Nitrogen unaccounted for was not proportional to N application, a greater fraction of the applied N being lost at the highest rate of N application (Table 5). Efficiency of N utilization by corn was high at low rates of fertilizer application. It is likely that, at a high rate of N application, a significant amount of applied N moved below the root zone and hence was not available to plants. High density of roots in plots with 200 kg N ha^{-1} probably promoted rapid denitrification.

In conclusion, this study has shown that fertilizer N use efficiency by corn in the Piedmont region was in the range of 43 to 57% of applied N. Although the efficiency of N utilization was the same from both NH_4^+ and NO_3^- sources, more N was lost from the field receiving NO_3^- than from NH_4^+. In the field plots receiving NH_4^+ a significant portion (up to 20%) was recovered in the organic fraction. Partitioning of labeled N in various plant parts showed that 9 to 17% was translocated to the seeds,

Table 5. Mass balance of labeled fertilizer N in soils planted with corn.

	$^{15}NH_4NO_3$	$NH_4{}^{15}NO_3$	Total
		kg N ha^{-1}	
	50 kg N ha^{-1}		
Plant	14.9	14.5	29.4
Soil			
Inorganic	1.2	0.2	1.4
Organic	14.5	9.4	23.9
Total N recovered	30.6	24.1	54.7
N unaccounted for	5.6	0.9	4.7
	100 kg N ha^{-1}		
Plant	24.1	28.9	53.0
Soil			
Inorganic	0.9	1.9	2.8
Organic	19.5	10.3	29.8
Total N recovered	44.5	41.1	85.6
N unaccounted for	5.5	8.9	14.4
	200 kg N ha^{-1}		
Plant	44.9	45.7	90.6
Soil			
Inorganic	2.8	0.7	3.5
Organic	18.7	5.4	24.1
Total N recovered	66.4	51.8	118.2
N unaccounted for	33.6	48.2	81.8

6 to 12% recovered in leaves, ≈6% in stem, <2% in ear leaf, <0.6% in tassel, 3 to 5% in the cob, and 3 to 5% in the weeds. The N unaccounted for in the soil–plant system was about threefold higher when fertilizer N application was increased from 100 to 200 kg N ha^{-1}.

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