

Fate of Nitrogen-15 in a Long-Term Nitrogen Rate Study: II. Nitrogen Uptake Efficiency

W. B. Stevens,* R. G. Hoefl, and R. L. Mulvaney

ABSTRACT

Increased fertilizer N uptake efficiency (FNUE) leads to more economical corn (*Zea mays* L.) production and lower environmental impact. Excessive N application reduces FNUE and may affect subsequent crop response through its influence on NO₃-N carryover and the amount of readily mineralizable organic N in the soil. Our objective was to determine how prior fertilizer N application rate affects (i) grain yield and agronomic optimum N rate, (ii) contributions of fertilizer- and soil-derived N to N uptake, and (iii) FNUE. Labeled ¹⁵NH₄¹⁵NO₃ was applied at 0, 67, 134, 201, or 268 kg N ha⁻¹ to subplots within a continuous corn long-term N rate study. Estimates of FNUE were higher by the difference method (49–69%) than with the isotope (¹⁵N) method (31–37%), and different trends were observed with each method as N application rate increased. The disparity between methods is consistent with a differential effect of long-term N application rate on mineralization–immobilization. Recovery of labeled N from the plant–soil system ranged from 71% at the 67 kg ha⁻¹ N application rate to 64% at the 201 kg ha⁻¹ application rate. Fertilizer N accounted for an increasing proportion of crop N uptake as the N rate was increased, but soil N uptake was always more extensive, accounting for 54 to 83% of total plant N. Crop uptake of labeled N during the second growing season after ¹⁵N application ranged from 2.2 kg ha⁻¹ with the lowest N rate to 7.8 kg ha⁻¹ with the two highest rates.

EFFICIENT USE of fertilizer N is becoming increasingly important in modern corn production due to rising costs associated with N fertilizer production and growing concern about NO₃ contamination of ground and surface waters. Interest in improving utilization of fertilizer N by crops has been particularly widespread in the Upper Mississippi River Basin (UMRB), which has been estimated to contribute 39% of the total N delivered to the Gulf of Mexico (Alexander et al., 1997). Moreover, continuous corn production, which is common in the UMRB, has been shown to contribute greater amounts of N to waterways than other common cropping systems (Weed and Kanwar, 1996; Randall et al., 1997). Research efforts during the past several decades have identified management and environmental factors that influence FNUE, and several mitigating strategies have been proposed (Di and Cameron, 2002; Dinnes et al., 2002). One commonly recommended approach is to avoid excessive N applications, which have repeatedly been shown to promote NO₃ loss by leaching (Jolley

and Pierre, 1977; Di and Cameron, 2000). The effectiveness of this strategy was demonstrated by data presented in a companion paper (Stevens et al., 2005) where post-harvest profile concentrations of mineral N were reported to have increased by 195 to 373% when N applications exceeded the long-term average optimum N rate.

Unfortunately, optimum N rates are difficult to predict for a particular site and year because of variability in soil moisture content and temperature, which greatly affect microbial N transformations (Franzluebbers et al., 1995; Weinhold and Halvorson, 1999). This difficulty is particularly serious in the UMRB, owing to a highly variable climate and the widespread occurrence of soils having high organic matter content. A yield-based approach is often used in this region to make fertilizer N recommendations for corn although an economic yield response is not always obtained. This was the case, for example, with 33 of 75 N response trials conducted in Illinois by Brown et al. (1993), which involved a number of nonresponsive sites that were not predictable on the basis of N credits associated with recent manure application or the presence of a previous forage legume. Such sites have been reported elsewhere in the north-central USA, including Wisconsin (Bundy and Malone, 1988; Bundy et al., 1992, 1999), Iowa (Blackmer et al., 1989), and Minnesota (Bundy et al., 1992; Schmitt and Randall, 1994). Recent work in Illinois has provided a chemical basis for their occurrence (Mulvaney et al., 2001) and has led to a new soil test as a means of detection (Khan et al., 2001).

Some of the disparities reported with traditional N recommendations may be related to the cumulative effect of past N management practices, such as repeated manure application or sustained fertilization with excessive amounts of chemically fixed N fertilizers. Indeed, studies at Rothamsted in England (Jenkinson, 1991) and the Morrow plots in Illinois (Odell et al., 1982) have shown that long-term application of N fertilizer at high rates can reduce subsequent N response. In a Wisconsin study by Motavalli et al. (1992) involving long-term N rates that were terminated after 25 yr, crop yield and spring soil NO₃ data were collected for each of the next 7 yr thereafter. Except for the presence of high levels of residual NO₃ in the first year following termination of the long-term N treatments, previous N rate did not affect spring concentrations of soil NO₃, whereas a sig-

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Abbreviations: AE, agronomic nitrogen use efficiency; ANI, added nitrogen interaction; FNUE, fertilizer nitrogen uptake efficiency; FNUE_{diff}, fertilizer nitrogen uptake efficiency calculated using the difference method; FNUE_{15N}, fertilizer nitrogen uptake efficiency calculated using the ¹⁵N isotope method; N_y, yield-based optimum N application rate; UMRB, Upper Mississippi River Basin; Y_{MAX}, predicted maximum yield; Y₀, grain yield produced on plots where no N was applied.

nificant effect usually was observed on grain yield and crop N uptake, which was attributed to more extensive mineralization of soil organic N with increased N fertilization. This conclusion is consistent with reports that higher N rates can enhance potential mineralization (El-Harris et al., 1983; Shen et al., 1989) by increasing the production of plant residues and/or promoting accumulation of organic N forms that are more mineralizable than native soil N (Shen et al., 1989).

While the literature clearly shows that previous N management practices can affect subsequent N response, the relationship is still poorly understood. More information is needed regarding the influence of long-term N fertilization history on the interaction of fertilizer- and soil-derived N pools in meeting the N requirement of corn. A replicated long-term N rate study in Illinois (Bullock and Bullock, 1994) provided an excellent opportunity to investigate this relationship using ^{15}N field research techniques. The objective of our study was to determine how different amounts of N fertilizer applied consistently from 1983 to 1993 would affect N response by subsequent corn crops. Specifically, effects of long-term N treatments on (i) grain yield and agronomic optimum N rate, (ii) contributions of fertilizer- and soil-derived N to plant N uptake through two growing seasons, and (iii) FNUE were investigated.

MATERIALS AND METHODS

Study Site Description and Experimental Design

The experiment was conducted from 1994 to 1996 on continuous corn plots of a long-term N rate study at the University of Illinois Northwest Research and Education Center located near Monmouth, IL. Experimental units within the long-term study, which is described by Bullock and Bullock (1994), have received annual urea applications of 0, 67, 134, 201, or 268 kg N ha⁻¹, such that any given plot has received the same rate of fertilizer N each year since the establishment of the study in 1983. The experiment was arranged in a randomized complete block design with three replications. Chemical characteristics and drainage classification of the soil (Muscatine silt loam; fine-silty, mixed, mesic Aquic Hapludolls) have been described previously (Stevens et al., 2005).

Treatment Application and Cultural Practices

Experimental units of the long-term N rate study measured 6.1 by 18.3 m and consisted of eight corn rows spaced 0.75 m apart. For the purposes of the present study, each experimental unit was divided into two 3.05- by 18.3-m areas, one for collection of grain yield data and the other for establishment of three 2.3- by 3.05-m ^{15}N -fertilized microplots (one in each study year). Immediately after planting hybrid corn seed (Dekalb 623, Monsanto, St. Louis, MO), double-labeled NH_4NO_3 (3.1 atom% ^{15}N) was applied in solution to each microplot using a CO_2 -pressurized spray boom, such that the application of labeled N provided the long-term N rate assigned to the surrounding plot. Unlabeled urea was applied to the remainder of each main plot at the appropriate application rate. Details regarding cultural practices, microplot layout, and ^{15}N fertilization have been described in the companion paper (Ste-

Table 1. Dates when plot operations were performed at Monmouth, IL.

Operation	1994	1995	1996
Harvest areas fertilized with urea	7 May	20 May	23 April
Corn planted	9 May	21 May	24 April
$^{15}\text{NH}_4^{15}\text{NO}_3$ applied to microplots	11 May	23 May	24 April
Plants thinned to uniform population	16 June	20 June	21 May
Microplots harvested	12 September	29 September	15 September
Main plots harvested	4 October	17 October	9 October

vens et al., 2005). Table 1 shows the dates when different plot operations were performed.

Sample Collection and Preparation

Whole plants were harvested at physiological maturity (Table 1) from a 1.5- by 1.5-m area in the center of each microplot, and the ears were separated from the stalks and leaves. Plants were harvested at this growth stage because N uptake and translocation are essentially complete (Ritchie and Hanway, 1982; Hunter et al., 1991), yet most leaves are still intact, thus allowing more complete recovery of aboveground biomass than if harvested at a typical harvest grain moisture level of <200 g kg⁻¹. Once removed from the plot, stalks and leaves were weighed and mechanically shredded so that a subsample could be collected. The subsample was weighed immediately and again after drying at 70°C to determine moisture content and then ground to pass through a 2-mm screen. The remainder of the stalks and leaves were returned to the microplot and evenly distributed. Ears were dried at 70°C and separated into grain and cobs, which were then weighed to determine dry weight and ground to pass through a 2-mm screen.

When the remaining grain had dried to a moisture content of <200 g kg⁻¹ (Table 1), main plots were harvested for yield using a mechanical harvester equipped with a scale for weighing grain collected from each plot. Crop residues from any given plot were deposited directly back onto the area whence they originated, taking care to avoid deposition of unlabeled residue on ^{15}N microplots. Weight of harvested grain was corrected to a moisture content of 155 g kg⁻¹. Following grain and dry matter harvest, soil samples were collected from each microplot and analyzed for total N and ^{15}N as described in Stevens et al. (2005).

Plant Sample Analysis

All stalk, cob, and grain samples were digested for total N determination using the salicylic acid-thiosulfate modification of the semimicro Kjeldahl procedure described by Bremner (1996), and the resulting digests were diluted to 25 mL with deionized water. A 10-mL aliquot was then diffused using the H_3BO_3 technique described by Stevens et al. (2000), and the diffused N was determined using an automatic titrator (Model 719 S Titrimo, Metrohm, Herisau, Switzerland) equipped with a combination pH microelectrode (Model MI-411, Microelectrodes, Inc., Bedford, NH). The titrated sample was acidified and processed as described previously (Stevens et al., 2000), so as to determine atom% ^{15}N through isotope-ratio analysis of a dried aliquot containing 50 to 200 μg of N using a mass spectrometer (Nuclide Model 3-60-RMS, Spectrumedix, State College, PA) equipped with an automated Rittenberg apparatus (Mulvaney, 1993).

Calculations

Both isotopic (^{15}N) and nonisotopic (difference method) techniques were applied in estimating FNUE. The isotopic estimate is based on recovery of labeled N in the aboveground portion of the plant and is calculated according to the following equations:

$$\text{Fertilizer N fraction } (N_f) = \frac{\text{atom } \% \text{ } ^{15}\text{N}_{\text{sample}} - \text{atom } \% \text{ } ^{15}\text{N}_{\text{check plot}}}{\text{atom } \% \text{ } ^{15}\text{N}_{\text{fertilizer}} - \text{atom } \% \text{ } ^{15}\text{N}_{\text{check plot}}} \quad [1]$$

$$\text{soil N uptake} = \text{total N uptake} - \text{fertilizer N uptake (kg ha}^{-1}) \quad [2]$$

$$\text{fertilizer N uptake} = N_f \times \text{total N uptake (kg ha}^{-1}) \quad [3]$$

$$\text{FNUE}_{15\text{N}} (\%) = \frac{\text{fertilizer N uptake (kg ha}^{-1})}{\text{fertilizer N applied (kg ha}^{-1})} \times 100 \quad [4]$$

where $\text{FNUE}_{15\text{N}}$ is fertilizer N uptake efficiency calculated using the ^{15}N isotope method. The difference method assumes that N fertilization has no effect on plant uptake of soil N, which is estimated from the amount of N contained in the aboveground portion of the crop grown on the unfertilized check plot. The efficiency of fertilizer N uptake is thereby calculated as

$$\text{FNUE}_{\text{diff}} (\%) = \frac{\text{N uptake}_{\text{fertilized plot}} - \text{N uptake}_{\text{unfertilized plot}}}{\text{fertilizer N applied}} \times 100 \quad [5]$$

Agronomic N use efficiency (AE) was estimated using the following equations:

$$\text{AE (kg kg}^{-1}) = \frac{\text{kg grain}}{\text{kg N applied}} \quad [6]$$

$$\frac{N_Y}{Y_{\text{MAX}}} (\text{kg Mg}^{-1}) = \frac{\text{agronomic optimum N application rate (kg ha}^{-1})}{\text{maximum grain yield (Mg ha}^{-1})} \quad [7]$$

where N_Y is yield-based optimum N application rate and Y_{MAX} is predicted maximum yield.

Statistical Analysis

Statistical analysis was accomplished using the SAS GLM, REG, CORR, and NLIN procedures (SAS Inst., 1998). Differences among treatment means were separated using Fisher's least significant difference procedure. Linear and quadratic trends were identified using standard single degree-of-freedom hypothesis-testing procedures. Optimum N rates were calculated as an estimate of the minimum N rate that would give the maximum yield. When the yield response to N was quadratic or linear and yield increased significantly throughout the entire range of N fertilizer rates, the highest N rate was assumed to be the optimum. When the yield response to added fertilizer N reached a plateau, the data were fitted to a quadratic-plus-plateau model (Bullock and Bullock, 1994), and the optimum N rate was identified as coinciding with

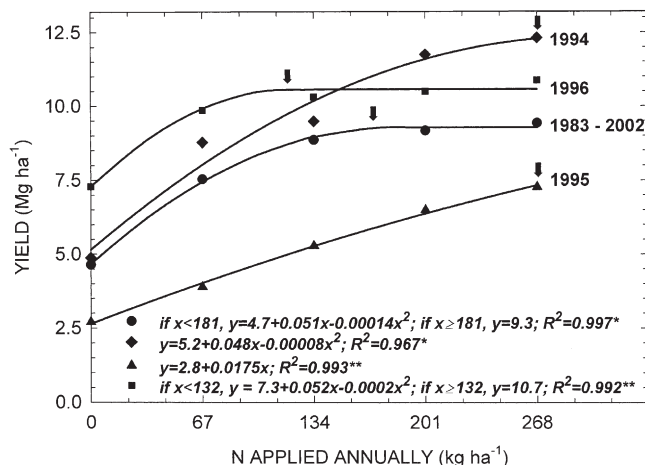


Fig. 1. The effect of long-term N application rate on grain yield in continuous corn production at Monmouth, IL. Arrows indicate the yield-based optimum N application rate (N_Y) at which maximum yield (Y_{MAX}) was obtained. The equation for each line is shown, preceded by the symbol corresponding to the specific data set. Symbols indicating statistical significance of the models follow each R^2 value, with * and ** indicating $P < 0.05$ and 0.01 , respectively. N_Y and Y_{MAX} values are listed in Table 2.

the transition from the quadratic to the plateau portion of the model.

RESULTS AND DISCUSSION

Yield

The relationship between N application rate and 20-yr average grain yield was best described by a quadratic-plus-plateau response model with a Y_{MAX} of 9.3 Mg ha^{-1} produced by a N_Y of 181 kg N ha^{-1} (Fig. 1). Excluding the 1988 and 1992 growing seasons when moisture stress prevented a yield response to N fertilization, yearly N_Y values varied widely from a low of 125 kg N ha^{-1} in 1996 to a high of 268 kg N ha^{-1} (the highest N application rate) in 1994 and 1995 (Table 2). The dramatic response to N fertilization observed in 1994 is not surprising, considering the grain yield produced that year was greater than at any other time during the 20-yr study period and very little mineral N remained in the soil profile in the spring of 1994 due to record precipitation in 1993 (Stevens et al., 2005). Conversely, grain production in 1995 was among the lowest recorded, owing to above-average precipitation early in the growing season that presumably limited yield potential and promoted N loss through denitrification and leaching.

Grain yield and supplemental N requirements within the 3-yr ^{15}N study period (1994 to 1996) also varied widely. In 1996, Y_{MAX} was slightly higher and N_Y was 31% lower than the corresponding 20-yr averages, whereas in 1994 and 1995, a value could not be calculated for N_Y because grain yield response to N fertilization increased throughout the range of N treatments. The implication is that fertilizer N use efficiency also varied significantly among the three growing seasons.

The amount of grain produced relative to the amount of fertilizer N applied may be used to estimate fertilizer use efficiency. The ratio of grain produced to N fertilizer

Table 2. Variation over time of check plot yield (Y_0), predicted maximum yield (Y_{MAX}), and yield-based optimum N application rate (N_Y) in continuous corn production at Monmouth, IL.

Year	Y_0	Y_{MAX}	N_Y †	N_Y/Y_{MAX}	AE‡
	Mg ha ⁻¹		kg N ha ⁻¹	kg N Mg grain ⁻¹	
1983	5.0	9.2	141	15.3	65.2
1984	5.2	10.8	217	20.1	49.8
1985	5.5	11.3	211	18.7	53.6
1986	4.4	10.9	198	18.2	55.1
1987	5.1	10.4	179	17.2	58.1
1988	3.4	4.1§	0	NA¶	0
1989	4.0	6.8	139	20.4	48.9
1990	5.8	11.3	152	13.5	74.3
1991	3.4	8.2	130	15.9	63.1
1992	3.4	5.3§	0	NA	0
1993	2.6	8.3	153	18.4	54.2
1994	4.9	12.4	268#	21.6	46.3
1995	2.7	7.4	268#	36.2	27.6
1996	7.3	10.5	125	11.9	84.0
1997	1.6	12.1	164	13.6	73.8
1998	6.0	12.1	220	18.2	55.0
1999	5.2	10.3	149	14.5	69.1
2000	5.6	10.7	144	13.5	74.3
2001	7.0	11.8	192	16.3	61.5
2002	4.7	6.5	134	20.6	48.5
Average††	4.6	9.3	181	19.5	51.4

† The lowest N rate that produced the maximum yield according to the quadratic-plus-plateau model.

‡ AE = agronomic N use efficiency; calculated using Eq. [6].

§ Yield was not significantly affected by N application rate, so values are yield means rather than predicted Y_{MAX} .

¶ NA = not applicable.

The response function did not reach a plateau, so Y_{MAX} and N_Y were assumed to be those values at the highest N application rate.

†† The average Y_{MAX} and average N_Y were derived from the quadratic-plus-plateau model fit to the 20-yr average yields.

applied (kg grain/kg N) has been defined as agronomic efficiency (AE) (Novoa and Loomis, 1981). Agronomic efficiency at N_Y is presented for each year in Table 2 and was calculated using Eq. [6]. In 1990, 1996, 1997, and 2000, the AE was well above the 20-yr average of 51.4 kg grain kg N⁻¹, indicating a more efficient use of fertilizer N during those four growing seasons. With the exception of 2000, this effect may have been related to an increase in N carryover from the previous year when poor grain yields limited utilization of fertilizer N. Profile data reported by Stevens et al. (2005) for this site show that preplant concentrations of inorganic N in 1996 averaged 48% higher than in 1994 and 7% higher than in 1995.

The inverse of AE can also provide an estimate of fertilizer N utilization. This ratio is presented in Table 2 as N_Y/Y_{MAX} and was calculated using Eq. [7]. While the same conclusions regarding fertilizer N use efficiency may be drawn from the N_Y/Y_{MAX} ratio as were drawn from AE, the data may also be used to evaluate the N requirement factor used in the University of Illinois N recommendation algorithm (Hoefl, 2000). The value of 21.4 kg N Mg grain⁻¹ used in the algorithm compares favorably with the 20-yr average N_Y/Y_{MAX} ratio of 19.5 kg N Mg grain⁻¹ (Table 2) but does overestimate the required amount of N by 9.7% for this study location. One weakness of evaluating or predicting N fertilizer application rates using either N_Y/Y_{MAX} or AE is that mineralization of soil N is ignored. Based on the potential for N mineralization to greatly influence N response (Mulvaney et al., 2001) and the spatial variability of N mineralization, accurate site-specific N recommendations are difficult to obtain using this approach.

Grain yield produced on plots where no N was applied (Y_0) has been used to estimate the amount of soil N

mineralized in fertilized plots and thus should relate to optimum N application rate (N_Y) and utilization of fertilizer N as indicated by AE; however, there was no significant correlation between Y_0 and N_Y or Y_0 and AE (Table 3). This poor relationship is not unexpected because, as concluded in our companion paper (Stevens et al., 2005), plots in this long-term study that have received no N fertilizer for a 20-yr period cannot be expected to have the same mineralization rate as plots that have received annual N fertilizer applications during that same period. Even though Y_0 was not correlated with N_Y , there was a significant relationship between Y_0 and Y_{MAX} . This underscores the fact that N mineralization, while important, is only one of several factors that influence yield. Precipitation (both amount and temporal distribution), temperature, pest levels, etc., vary from year to year, and each can have a considerable influence on yield. Since the influence of these factors would likely affect Y_0 and Y_{MAX} proportionally, one would expect these two variables to be correlated. The strongest correlations ($r = 0.6926$ and $r = 0.7258$) were observed between Y_{MAX} and N_Y and Y_{MAX} and AE (Table 3),

Table 3. Correlation coefficients (r) and probability values (p) showing relationships among yield and N use efficiency variables for Monmouth long-term N plots, Monmouth, IL (1983–2002).

	Y_{MAX} †	N_Y ‡	N_Y/Y_{MAX}	AE§
Y_0 ¶	$r = 0.4735$ $p = 0.035$	$r = 0.1913$ $p = 0.419$	$r = -0.3833$ $p = 0.116$	$r = 0.4347$ $p = 0.056$
Y_{MAX}		$r = 0.6926$ $p = 0.001$	$r = -0.4272$ $p = 0.077$	$r = 0.7258$ $p = <0.001$
N_Y			$r = 0.6813$ $p = 0.002$	$r = 0.4880$ $p = 0.074$

† Y_{MAX} = predicted maximum yield.

‡ N_Y = yield-based optimum N application rate.

§ AE = agronomic efficiency [kg grain (kg N applied)⁻¹] at N_Y .

¶ Y_0 = yield with no added N.

indicating that both N_Y and AE increase with increasing yield potential. These relationships are not surprising, but the positive relationship between AE and yield potential emphasizes that environmental conditions that favor greater yield also lead to more efficient utilization of fertilizer N, presumably because of lower N losses and/or greater mineralization of soil organic N.

Fertilizer Nitrogen Uptake Efficiency

Isotope Method

Recovery of labeled N in aboveground plant biomass increased linearly with the rate of N applied (Table 4). While recovery differed among the 3 yr studied, the $N \times$ year interaction was not significant ($P < 0.05$), so only main-effect means are shown. The lower recovery of fertilizer N in 1995 as compared with 1994 was probably due to poor crop growth associated with hot, dry conditions late in the growing season and/or cool, wet spring weather in 1995 compared with unusually favorable conditions in 1994. Of the total fertilizer N recovered in the plant, about two-thirds was present in the grain, one-third in the stalk, and $<3\%$ in the cobs. These results are consistent with a previous study by Jokela and Randall (1997), in which 65 to 75% of the fertilizer N assimilated by corn was recovered in the grain.

Fertilizer-derived N accounted for a higher percentage of total N uptake as N application rate increased (Fig. 2), yet $FNUE_{15N}$ remained relatively constant (Table 4). The latter finding is somewhat surprising in view of previous reports that increases in N application decrease $FNUE_{15N}$ (Sanchez and Blackmer, 1988; Reddy and Reddy, 1993; Jokela and Randall, 1997) but is confirmed by the strong linear relationship observed between the uptake of fertilizer-derived N and the amount of fertilizer N applied (Fig. 2).

The contribution of soil-derived N (i.e., residual mineral N, mineralized organic N, and displaceable clay-fixed NH_4-N) to crop N uptake is also shown in Fig. 2. For ^{15}N -fertilized plots, the soil supplied 54 to 83% of the total N assimilated by the corn crop with the percentage

decreasing as the long-term N application rate increased. Yet N fertilization history had the opposite effect on the absolute uptake of soil-derived N, which increased from 74 kg N ha^{-1} for the unfertilized check plot (estimated from total N uptake) to 120 kg N ha^{-1} when 201 kg ha^{-1} of fertilizer N was applied. There was a quadratic relationship between uptake of soil-derived N and long-term N application rate, with a slight decrease between 201 and 268 kg N ha^{-1} . This decrease likely did not occur because of reduced mineralization but because plant N demand was probably exceeded by the supply of mineral N at application rates beyond the optimum (181 kg ha^{-1}). If this were the case, one would expect an accumulation of unutilized soil-derived mineral N in the profile of plots receiving 201 or 268 kg N ha^{-1} , which was indeed reported by Stevens et al. (2005).

Figure 2 illustrates an important distinction between crop uptake of soil- and fertilizer-derived N, which at least partially explains the different effects of N application history on $FNUE$. The linear effect of N rate on the uptake of fertilizer N is as might be expected following a pulse input of readily available mineral N, provided that leaching and denitrification losses were limited. Fertilization likewise promoted the uptake of soil-derived N, but the trend was quadratic rather than linear, presumably because availability of N to the plants was limited by the need for mineralization, with the result that uptake of fertilizer N would have been increasingly favored with higher N rates. Owing to an abundance of fertilizer-derived mineral N, this preference would have been most marked early in the growing season and would have decreased with time as fertilizer N was assimilated and soil N mineralized. In a study by Omay et al. (1998), uptake of labeled fertilizer N by corn was found to be essentially complete at tasseling (VT growth stage), with only soil N being taken up thereafter.

The results in Fig. 2 also demonstrate the critical role played by mineralization in supplying the N required by corn. Even with the highest N rate studied (268 kg ha^{-1}), uptake of fertilizer N was exceeded by uptake of soil N. This finding is consistent with many previous

Table 4. Recovery of fertilizer N by the aboveground portion of corn plants during the first growing season after application of $^{15}NH_4^{15}NO_3$ at Monmouth, IL, 1994–1996.

N applied	Recovery of fertilizer N†				Uptake efficiency‡	
	Grain	Stalks	Cobs	Total	$FNUE_{15N}$	$FNUE_{diff}$
	kg ha^{-1}				recovery, %	
67	14.5 d	5.7 c	0.5 c	20.7 d	31 a	69 a
134	31.6 c	12.7 c	0.9 c	45.3 c	34 a	60 b
201	52.0 b	21.1 b	1.4 b	74.6 b	37 a	59 b
268	63.1 a	30.1 a	1.6 a	94.9 a	35 a	49 c
N rate effect	L**§	L**	L*	L**	NS	L*
Year effect						
1994	47.2 a	17.9 ab	1.7 a	66.3 a	39 a	67 a
1995	33.1 b	16.1 b	1.2 b	50.1 b	28 b	54 b
1996	40.7 a	18.2 a	1.6 a	60.2 a	36 ab	57 ab

* Indicates statistical significance at the 0.05 probability level.

** Indicates statistical significance at the 0.01 probability level.

† For a given effect, means within a column followed by the same letter are not significantly different ($P < 0.05$). ANOVA showed no significant N rate \times year interaction.

‡ $FNUE_{15N}$ = fertilizer N uptake efficiency calculated using Eq. [4] for the ^{15}N method; $FNUE_{diff}$ = fertilizer N uptake efficiency calculated using Eq. [5] for the difference method.

§ L = linear effect of N rate on the corresponding dependent variable.

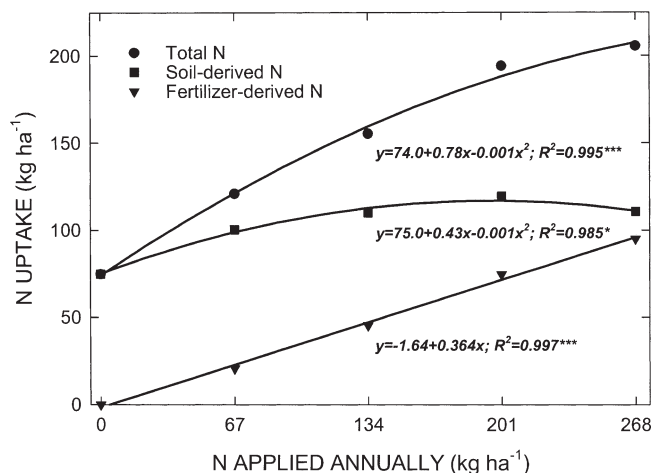


Fig. 2. Relationships between N fertilizer application rate and uptake of total N, fertilizer-derived (¹⁵N-labeled) N, and soil-derived (non-labeled) N during the growing season following application of labeled N (1994–1996, Monmouth, IL). The equation for each line is shown below its corresponding curve. Symbols indicating statistical significance of the models follow each R^2 value, with *, **, and *** indicating $P < 0.05$, 0.01 , and 0.001 , respectively.

studies using ¹⁵N to estimate fertilizer N recovery by corn (e.g., Bigeriego et al., 1979; Olson, 1980; Kitur et al., 1984; Torbert et al., 1993; Jokela and Randall, 1997; Omay et al., 1998), in which the majority of plant N has often been found to originate from the soil. Surprisingly, mineralization has sometimes been ignored in developing models to describe the relationship between agricultural N fertilizer management and surface water N loading (e.g., Howarth et al., 1996; David and Gentry, 2000).

Difference Method

As with the isotope method, estimates of FNUE using the difference method ($FNUE_{diff}$) decreased as the amount of N applied annually increased and were highest in 1994 when growing conditions were most favorable (Table 4). The $FNUE_{diff}$ estimates were substantially higher than those based on the ¹⁵N method, suggesting the occurrence of a positive added N interaction (ANI). The observed increase in plant uptake of soil-derived (unlabeled) N with increasing N application (Fig. 2) also supports this conclusion. An ANI is defined by Jenkinson et al. (1985) as any effect that the addition of N to soil may have on N already present in a given soil N pool. This effect can either be “real” or “apparent” depending on whether the addition of N causes (i) a change in the processes that move N into and out of a particular pool (real) or (ii) the displacement or substitution of N already present in a particular pool by N that is added (apparent). Examples of real ANIs include (i) stimulation of microbial growth by added fertilizer N (Westerman and Kurtz, 1973), (ii) osmotic effects (Broadbent and Nakashima, 1971), and (iii) increased root growth in fertilized vs. unfertilized soils (Olson and Swallow, 1984). Jenkinson et al. (1985) concluded based on a review of literature that ANIs caused by these processes are likely to be small under most circumstances. They further concluded that the process most likely to give rise to an apparent ANI in the presence

Table 5. Recovery of residual fertilizer N by corn plants 1 yr after application of ¹⁵NH₄¹⁵NO₃ at Monmouth, IL. Data are averages of 1995 and 1996 yearly means.

N rate	Residual fertilizer N [†]	Crop uptake of residual fertilizer N [‡]	Recovery of residual fertilizer N
	kg ha ⁻¹		%
67	36	2.2 b	6
134	57	3.4 b	6
201	83	7.8 a	9
268	104	7.8 a	8

[†] Residual fertilizer N is the labeled N present in the spring of the second growing season calculated as the sum of labeled soil organic N, labeled soil inorganic N, and labeled N in the previous crop's residue.

[‡] Means followed by the same letter are not significantly different ($P < 0.05$).

of plants is pool substitution resulting from immobilization of fertilizer N. The magnitude of this apparent ANI is equal to the quantity of N immobilized. However, as noted in our companion paper (Stevens et al., 2005), differences among treatments with regard to postharvest levels of soil-derived residual inorganic N were substantially greater than differences in the amount of fertilizer-derived N immobilized. When soil-derived N recovered in the plant (Fig. 2) is also considered, this discrepancy is even greater, indicating that processes other than immobilization-driven pool substitution contributed to the increase in soil-derived N uptake that occurred as the annual N application rate increased from 0 to 201 kg N ha⁻¹. We conclude that the effects observed in this study were at least partially the result of differences among long-term N treatments in the mineralization of N from crop residues and soil organic matter. These results also suggest that it may not be appropriate to use the difference method to estimate FNUE under the conditions of this study because N mineralization in plots receiving no N over a 14-yr period cannot be expected to be the same as in plots with a history of annual N application over the same time span. This is in agreement with Schindler and Knighton (1999), who suggested that the difference method is most appropriate for fertilizer N experiments that are newly established and where variability among plots in soil N availability is minimal.

Recovery of Fertilizer Nitrogen during the Second Growing Season

In 1995 and 1996, corn was grown on the previous year's microplot areas to evaluate recovery of residual fertilizer-derived N from the soil and the previous crop's residue. From 6 to 9% of the labeled fertilizer N was recovered in the second year's crop, representing 2 to 8 kg N ha⁻¹ if no apparent ANI is assumed to have occurred (Table 5). While this is a relatively small effect for a single fertilizer application, there is an implication that numerous annual fertilizer applications could substantially reduce the response of corn to N fertilization.

Further examination of Table 5 reveals that crop uptake of residual fertilizer N was significantly greater with 201 or 268 than with 67 or 134 kg N ha⁻¹. This difference, which was still evident even when recovery was expressed as a percentage of the fertilizer N applied, can probably be attributed to several factors. First, data

reported by Stevens et al. (2005) show that plots receiving the two highest N rates contained significantly more fertilizer-derived organic N in the spring as compared with the others. This finding suggests a more extensive release of inorganic N through mineralization of newly formed organic N, which was shown by Shen et al. (1989) to be several times more mineralizable than older forms of soil N. Second, as N application rate increased, a larger quantity of fertilizer-derived N was returned to the plot with the stalks and cobs (Table 4). A portion of this N would have become available to the succeeding crop as inorganic NO_3 leached from the residue and as organic N compounds were mineralized. A study by Power et al. (1986) implicates the latter process as the major source of residual N availability; however, their work differed from ours in that the residue had been produced with low fertilizer N inputs and was not incorporated. Finally, the greater uptake of fertilizer-derived N observed with 201 or 268 kg N ha^{-1} may have been due in part to accumulation of residual inorganic N. As reported by Stevens et al. (2005), essentially no labeled inorganic N was detected in plots receiving 67 or 134 kg N ha^{-1} , whereas recoveries in this form ranged from 35 to 54 kg N ha^{-1} with the two highest N rates.

Mass Balance of Fertilizer Nitrogen

As summarized by Table 6, from 64 to 71% of the fertilizer N applied was recovered in the entire plant–soil system. When expressed in terms of percentage recovery, the N deficit was unexpectedly consistent, ranging from 29% when 67 kg N ha^{-1} was applied to

Table 6. Mass balance of ^{15}N -labeled fertilizer N as affected by long-term N application rate in continuous corn production. Values are 3-yr means of data collected annually at Monmouth, IL, from 1994 to 1996.

Form of N	Labeled N recovered	
	kg ha^{-1}	percentage of applied, % 67 kg N ha^{-1}
Plant	20.7	31
Soil		
Inorganic	2.8	4
Organic	24.3	36
Total	47.8	71
N unaccounted for	19.2	29
		134 kg N ha^{-1}
Plant	45.2	34
Soil		
Inorganic	3.9	3
Organic	39.8	30
Total	88.9	66
N unaccounted for	45.1	34
		201 kg N ha^{-1}
Plant	74.6	37
Soil		
Inorganic	13.2	7
Organic	41.8	21
Total	129.6	64
N unaccounted for	71.4	36
		268 kg N ha^{-1}
Plant	94.9	35
Soil		
Inorganic	30.7	11
Organic	48.6	18
Total	174.2	65
N unaccounted for	93.8	35

36% with 201 kg N ha^{-1} . In terms of absolute N loss, the deficit increased linearly with increasing N application rate ($R^2 = 0.999$), such that for each kilogram of fertilizer N applied, 0.38 kg was lost. These losses presumably occurred by leaching and denitrification and perhaps also through N volatilization from the plants.

SUMMARY AND CONCLUSIONS

Hybrid corn yield increased throughout the range of fertilizer N rates in 1994 and 1995, but in 1996, the yield reached a plateau at about 125 kg N ha^{-1} . Over the 20 yr since the initiation of the N-rate study at Monmouth, the average yield plateau has occurred at 181 kg N ha^{-1} . Approximately 40% of the labeled fertilizer N applied was recovered in a very favorable growing season (1994) as compared with 28% in a relatively poor growing season (1995) and 36% under intermediate conditions (1996). When estimated using the difference method, uptake of fertilizer N showed a similar pattern over the 3-yr study period but was substantially higher (54–67%) than when estimated with the isotope (^{15}N) method. During the second growing season after application of ^{15}N -labeled fertilizer, plant recoveries of labeled N ranged from 6 to 9% of the fertilizer N originally applied and were most extensive with the two highest N rates used (201 and 268 kg N ha^{-1}). These data suggest that mineralization of residual N is increased when fertilizer N has been applied previously at a high rate and that a series of such applications may lead to a substantial decrease in crop response to subsequent N fertilization while promoting N losses. Our study provides further evidence of the need to avoid excessive N fertilization.

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