Published November, 2004

Nitrogen Balance as Affected by Application Time and Nitrogen Fertilizer Rate in Irrigated No-Tillage Maize

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ABSTRACT

High N requirements of no-tillage maize (*Zea mays* L.) make it imperative to develop management strategies that optimize crop production and N use efficiency (NUE). A 4-yr field experiment was conducted at Balcarce (37°45′ S, 58°18′ W), Argentina, on a Typic Argiudoll and a Petrocalcic Paleudoll. The objective was to evaluate the effect of urea rate (0, 70, 140, and 210 kg N ha^{-1}) at planting (FPL) or six-leaf stage (FV6) on NH_3 volatilization, denitrification, soil residual nitrate, soil microbial biomass N (MBN), N uptake, grain yield, and unaccounted N (UN). Grain yield was 10.5 and 11.2 Mg ha^{-1}, and N uptake at physiological maturity was 168 and 192 kg N ha^{-1} (average of N rates) for FPL and FV6, respectively. Gaseous N losses ranged from 7.6 to 13.8% of applied N and were not affected by the fertilizer time. Relative to unfertilized control, fertilized treatments increased MBN (13.4 kg N ha^{-1}) similarly for both fertilization times. For FPL, UN was 55, 69, 86, and 103 kg N ha^{-1} for 0, 70, 140, and 210 kg N ha^{-1}, respectively. For FV6, UN was 55, 46, 49, and 34 kg N ha^{-1} for 0, 70, 140, and 210 kg N ha^{-1}, respectively. The losses were attributed to nitrate (NO_3) leaching. Results of this experiment show that high fertilizer NUE in combination with economically competitive grain yields can be obtained when N is applied at V6 because gaseous N losses are low (less than 13.8%) and NO_3 leaching would be reduced.

The southeastern portion of Buenos Aires province in Argentina has a temperate-humid climate and soils with high organic matter content. Nevertheless, during the last two decades, intensive cropping with conventional tillage (CT) has lead to a deterioration of soil physical and chemical properties. A reduction in soil organic matter in particular has lead to an increase in soil erosion and exacerbated N deficiency problems (Studdert and Echeverría, 2000). Producers have responded to this problem by increasing the use of no-tillage (NT) production systems.

Compared with CT, NT systems in temperate-humid areas can change the soil environment and consequently can decrease N mineralization and nitrification while increasing N immobilization, denitrification, and/or leaching (Fox and Bandel, 1986). A greater immobilization and decrease in N mineralization in soils under NT leads to an increase in the soil organic N (Meisinger, 1984). During a period of 7 yr, NT increased soil organic C (3.8%), and consequently organic N, in soil surface (0–20 cm), with respect to CT at Balcarce (Studdert and Echeverría, 2002). Therefore, fertilizer N requirement of maize under NT is greater than CT (Meisinger et al., 1985). Consequently, it is very important to increase the recovery of applied N and maize production in NT systems if we are to increase the economic return to the farmer and reduce potential for NO_3^- leaching or other adverse environmental impacts.

Nitrogen balances have been used to estimate the UN in a given agricultural production system (Legg and Meisinger, 1982). A number of ^{15}N field balances, in maize crops under CT fertilized at planting time, have shown N deficits ranging from 15 to 27% of the applied N, and it is presumed that the deficit reflects denitrification losses (Olson, 1980; Reddy and Reddy, 1993; Bigeriego et al., 1979). Application at V6 results in losses of applied N in the range of only 6.7% (Bigeriego et al., 1979).

In the southeastern portion of Buenos Aires province, fertilizer N recovery in irrigated maize under NT is higher for FV6 than FPL (Sainz Rozas et al., 1997a, 1999). Nitrogen recovery by the maize crop was 71 and 58% (average of N rates) for FV6 and for FPL, respectively (Sainz Rozas et al., 1997a). For FPL, these authors reported denitrification losses ranging from 2.6 to 5.5% of applied N, for N rates of 210 and 70 kg ha^{-1}, respectively. For FV6, theses losses ranged from 0.4 to 1% of applied N for the same N rates. Mean volatilization loss was 4.3 and 9.2% (average of N rates) of applied N for FPL and FV6, respectively (Sainz Rozas et al., 1999, 2001). Therefore, gaseous N losses (volatilization plus denitrification losses) were similar for both fertilization times, and it indicates that another mechanism of N loss, other than denitrification, decreased N recovery for FPL with respect to FV6.

Jokela and Randall (1997) reported greater N immobilization in organic forms when N fertilizer was applied at planting compared with V6. Nevertheless, the relative importance of this pool when calculating a whole-crop N balance has not been reported in soils under NT in Argentina. On the other hand, at Balcarce, in a maize crop under CT fertilized at planting with 200 kg N ha^{-1}, Costa et al. (2003) reported leaching losses of 66.5 kg N ha^{-1}. However, the magnitude of these losses has not been reported for irrigated maize crop under NT in the Argentina.

There are reports in the literature where partial aspects of N cycle (N uptake, mineral N, volatilization, and denitrification losses) as affected by N rate and application time have been studied (Sainz Rozas et al., 1999, 2001), but up to this point in time, this information has not been incorporated into an N balance equation. An N balance using part of a data set (Sainz Rozas et

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Published in Agron. J. 96:1622–1631 (2004).
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Abbreviations: CET, crop evapotranspiration; C_L N_2O-N, cumulative nitrous oxide nitrogen losses; CT, conventional tillage; FPL, fertilization at planting time; FV6, fertilization at six-leaf stage; MBN, microbial biomass nitrogen; NT, no-tillage; NUE, nitrogen use efficiency; UN, unaccounted nitrogen.

al., 1999, 2001) would assist in the identification of the main N loss mechanism and would thus be of use when considering approaches to decrease N loss from these NT maize systems. The objective of this research was to estimate an N balance for continuous NT irrigated maize as a function of N rate and application time.

MATERIALS AND METHODS

The experiment was conducted in four growing seasons (1994–1995, 1995–1996, 1996–1997, and 1998–1999) at the Instituto Nacional de Tecnología Agropecuaria (INTA) Research Station, Balcarce (37°45′ S, 58°18′ W; 130 m above sea level; 870 mm mean annual rainfall; 13.7°C mean annual temperature), Buenos Aires, Argentina.

The experimental field contains a soil complex consisting of 90% fine, mixed, thermic Typic Argiudoll and 10% fine, illitic, thermic Petrocalcic Paleudoll (petrocalcic horizon was below 70 cm). The Typic Argiudoll has a loam texture at the surface layer (0- to 25-cm depth), loam to clay loam at subsurface layers (25- to 110-cm depth), and sandy loam below 110-cm depth (C horizon). Some soil characteristics determined at the time of planting are presented in Table 1.

The preceding crop of the first NT maize (1994) crop was soybean [*Glycine max* (L.) Merr.] that had been NT-planted into wheat (*Triticum aestivum* L.) stubble. Residue cover at planting for the first maize crop was 70%. In the following growing seasons, NT maize crops were planted on the same plots. Ground cover by maize residue ranged from 80 to 90%. Two single-cross maize hybrids were used: 'Dekalb 636' in the first two seasons (1994–1995 and 1995–1996) and 'Dekalb 639' in the 1996–1997 and 1998–1999 growing seasons. In the 1997–1998 growing season, maize crop without N was planted in all plots. Maize was planted during the first or second week of October each year. The distance between adjacent rows was 70 cm, and final plant population was 63 700, 75 000, 79 000, and 73 400 plants ha^{-1} for the first, second, third, and fourth year, respectively. Plots were 12 m long and four rows wide (2.8 m, 33.6 m^2) in the first 2 yr and five rows wide (3.5 m, 42.0 m^2) in the last 2 yr. Weeds and insects were chemically controlled with recommended products and rates. Plots were fertilized annually at planting with 20 kg P ha^{-1} and sprinkle-irrigated during high-water-requirement periods so that these production factors did not limit crop growth. In the 1996–1997 growing season, due to an error in the calculation for irrigation, available water for the crop in the month of January exceeded crop evapotranspiration (CET) by 60 mm. The CET was determined as the product between potential evapotranspiration (ET0) and crop coefficient (Kc). The ET0 was determined according to Penman (1948). The Kc (CET/ET$_0$) values are those reported for the area by Della Maggiora et al. (2000).

The experimental design was a factorial treatment arrangement with a control (0 N) in a randomized complete block. In the first year, the factorial arrangement was a control plus a 2 × 2: two N rates [70 and 140 kg N ha^{-1}] and two N fertilization times (planting and V6). In the second year and last year, the factorial arrangement was a control plus a 3 × 2: three N rates (70, 140, and 210 kg N ha^{-1}) and two N fertilization times (planting and V6). In the third year, the factorial arrangement was again a control plus a 2 × 2: two N rates (70 and 210 kg N ha^{-1}) and two N fertilization times (planting and V6). In all cases, urea was surface-broadcast.

Ammonia losses were evaluated during the 1994–1995 and 1995–1996 growing seasons for both fertilization times for the 0, 70, 140, and 210 kg N ha^{-1} rates, these results being part of a previous work (for more details about method of determination, see Sainz Rozas et al., 1999). A semiopen-static system (Nommik, 1973) was used to monitor NH$_3$ volatilization losses from the plots. It consisted of a polyvinyl chloride (PVC) 30-cm-diam. and 50-cm-height cylinder per experimental unit, containing two polyurethane sponges saturated with H$_2$SO$_4$ (0.5 *M*) to capture the NH$_3$. The sponges were changed every 24 h and washed with 1.5 L of deionized water. An aliquot of 25 mL was alkalinized with NaOH (40%), and NH$_3$–N was determined by microdistillation (Bremner and Keeney, 1966). In 1996–1997 and 1998–1999 growing seasons, denitrification losses were determined from plots that received 0, 70, and 210 kg N ha^{-1} at both fertilization times. Denitrification losses were generally estimated weekly or biweekly during the growing season. Rates of N$_2$O-N were accumulated from planting to flowering (R1) and from planting to physiological maturity (R6) in the 1996–1997 and 1998–1999 growing seasons, respectively. Denitrification rates (N$_2$O) under field conditions were estimated by the C$_2$H$_2$ inhibition method (Yoshinari et al., 1977). Intact soil cores (4.2 cm i.d. × 13 cm long) were randomly obtained from between-row soil in each plot using PVC cylinders. The cylinders were sealed with two rubber stoppers, the upper stopper having a septum. Ten percent (v/v) of the gas enclosed in the cylinder was replaced with an equivalent volume of acetylene. Then, cylinders were incubated outside the laboratory in an open, but shaded environment for a 24-h period, after which 10 mL of a gas sample was removed for analyses of N$_2$O-N concentration using a 5890 Series-II Hewlett-Packard gas chromatograph equipped with a ^{63}Ni electron-capture detector (for more details about method of determination, see Sainz Rozas et al., 2001). Denitrification losses for 140 kg N ha^{-1} were not determined in any growing season. Therefore, denitrification losses for this N rate were estimated from a relationships between denitrification losses and N rates (0, 70, and 210 kg N ha^{-1}) when this relationship was significant. If this relationship was not significant, denitrification losses from 140 kg N ha^{-1} resulted from an average between 70 and 210 kg N ha^{-1}.

Table 1. Selected surface soil (0–20 cm) characteristics at planting time for the experimental site.

Year	P†	pH‡	Organic C§
	mg kg^{-1}		g kg^{-1}
1994	15.2	5.8	32.0
1995	18.2	5.8	32.0
1996	23.0	5.8	32.0
1998	26.1	5.8	32.0

† Bray and Kurtz (1945).
‡ Determined with a glass electrode in a suspension of 1:2.5 soil/water ratio.
§ Walkley and Black (1934).

In each growing season, at planting time, soil samples were collected from the control plots to estimate the soil mineral N level. All treatment combinations were sampled at R6. Samples were collected at 0- to 5-, 5- to 20-, 20- to 40-, 40- to 60-, 60- to 80-, and 80- to 100-cm depths. Inorganic N was extracted from fresh samples with K$_2$SO$_4$ (0.5 *M*) and NO$_3^-$–N, and NH$_4^+$–N contents were determined by microdistillation (Bremner and Keeney, 1966).

In each experimental unit, soil N content in the MBN was determined at planting and at R6 stage by the chloroform fumigation–extraction technique (Brookes et al., 1985) at 0- to 5- and 5- to 20-cm soil depths. The results obtained in each depth were corrected by soil bulk density to transform them to kg N ha^{-1} to 20-cm soil depth. The MBN was calculated as:

$$\mathrm{MBN} = (F - \mathrm{NF})/\mathrm{kn}$$

where F = NH$_4$–N liberated by chloroform-fumigated sample, NF = NH$_4$–N liberated by the nonfumigated sample, and kn = 0.47 (Ferrari et al., 1992–1993). The fumigation was performed by placing each soil sample in a dryer and exposing it to chloroform vapors for 24 h. The organic and inorganic N was extracted with K$_2$SO$_4$ 0.5 *M* at a ratio of 1:4 (soil/K$_2$SO$_4$). Soil extracts (15 mL) of fumigated and nonfumigated soil samples were digested with 5 mL of concentrated H_2SO_4 and 0.3 mL of $CuSO_4$. Sample NH_4–N content was determined by microdistillation (Bremner and Keeney, 1966).

Each growing season, 10 maize plants were collected for determination of aboveground dry matter accumulation at the R6 growth stage. Plants were cut at ground level; separated into leaf blades, stalk plus sheaths plus tassel plus husks, and grain; and oven-dried, weighed, and milled to pass a 1-mm mesh. Reduced N was determined in each fraction by Method A (without salicylic acid modification) as reported by Nelson and Sommers (1973). Total N accumulated in each fraction was calculated as the product of its N concentration (dry weight basis) and dry weight. At maturity, 7.15 m of the two center rows of each experimental unit was hand-harvested to determine grain yield. All reported yields were corrected to 140 g kg^{-1} grain moisture content.

A whole-crop N balance approach (Meisinger, 1984) was used to estimate the UN in the soil–plant system, according to the following equations:

$$\mathrm{Nf} + N_{\mathrm{min}} + N_{\mathrm{sinb}} = N_{\mathrm{gr}} + N_{\mathrm{vol}} + N_{\mathrm{desn}} + N_{\mathrm{stover}} + N_{\mathrm{root}} + \Delta_{\mathrm{MBN}} + N_{\mathrm{sine}} + \mathrm{UN}$$

Solving for UN:

$$\mathrm{UN} = (\mathrm{Nf} + N_{\mathrm{min}} + N_{\mathrm{sinb}}) - (N_{\mathrm{gr}} + N_{\mathrm{vol}} + N_{\mathrm{desn}} + N_{\mathrm{stover}} + N_{\mathrm{root}} + \Delta_{\mathrm{MBN}} + N_{\mathrm{sine}})$$

where

Nf = fertilizer N input
N_{min} = N produced from mineralization
N_{sinb} = NO_3^-–N at the beginning growing season
N_{sine} = NO_3^-–N at the end of the growing season
N_{gr} = N removed in grain
N_{vol} = volatilization N losses
N_{desn} = cumulative N_2O-N losses
N_{stover} = stover returned N
N_{root} = root returned N
Δ_{MBN} = change in soil microbial biomass N content (0–20 cm) between physiological maturity (end) and planting (beg), i.e., $\Delta_{\mathrm{MBN}} = \mathrm{MBN}_{\mathrm{end}} - \mathrm{MBN}_{\mathrm{beg}}$

In the 1996–1997 and 1998–1999 growing seasons, the occurrence of a rainfall event (18 mm) shortly after FPL and FV6 (1 d after fertilization) prevented N volatilization (Fox et al., 1986), and therefore volatilization losses did not contribute to the UN.

Nitrogen mineralized during the growing season was determined using the model reported by Echeverría et al. (1994). This model integrates the potentially mineralizable N (N_0), N mineralization constant, soil temperature, and soil moisture at the 20-cm depth during the growing season. The N_0 was determined by anaerobic incubations of 14 d (Echeverría et al., 2000).

Nitrogen returned in crop root to the soil was estimated as a proportion of total N accumulated in aerial biomass. These values were 6.6 and 5.3% for control and fertilized treatments, respectively (Uhart and Andrade, 1995).

Treatment effects were evaluated by analysis of variance using the Statistical Analysis System (SAS) (SAS Inst., 1985). Following the *F* test in ANOVA, multiple comparison of means ($p < 0.05$) was conducted with a Fisher's protected least significant difference (LSD).

RESULTS AND DISCUSSION

Water availability did not limit maize yield in any growing season because rainfall and irrigation met CET, during the critical period for kernel number set (January) (Fig. 1). During the 1996–1997 growing season, the maize rough dwarf virus disease could have slightly af-

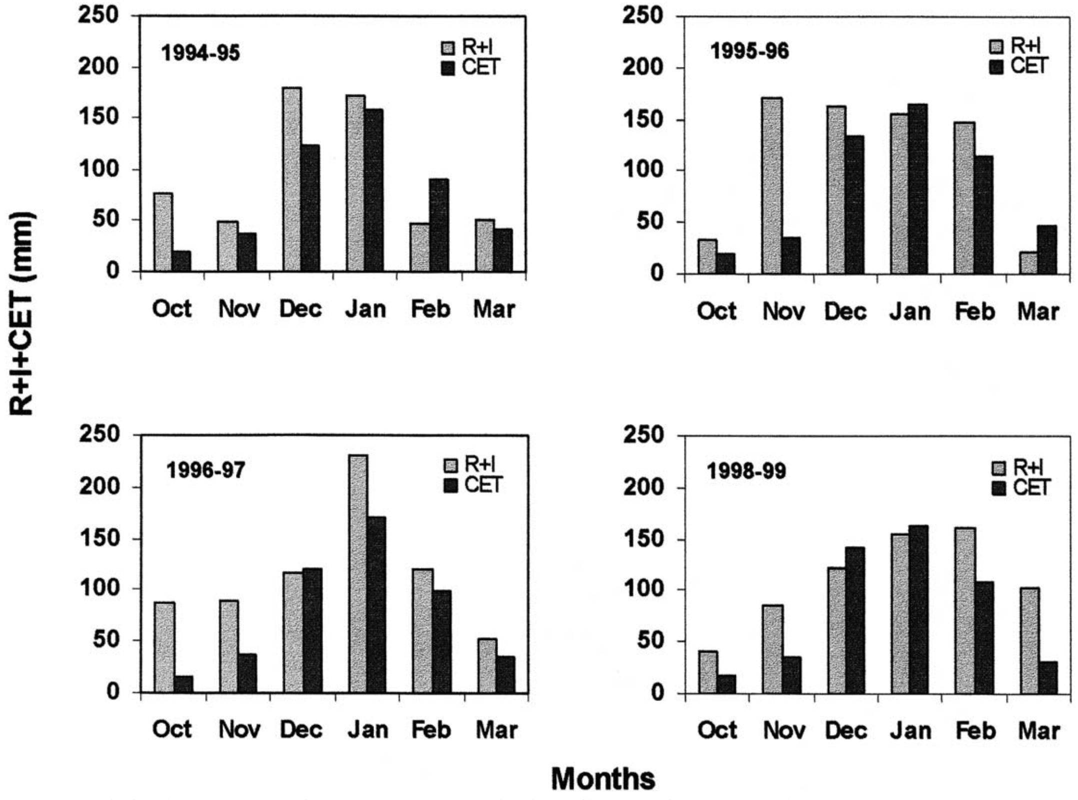

Fig. 1. Rainfall (R) plus irrigation (I) and maize crop evapotranspiration (CET) during the growing season.

Table 2. Monthly means of air mean temperature (T) and incident radiation (IR) for the 1994–1995, 1995–1996, 1996–1997, and 1998–1999 growing seasons.

	1994–1995		1995–1996		1996–1997		1998–1999	
Month	**T**	**IR**	**T**	**IR**	**T**	**IR**	**T**	**IR**
	°C	MJ m^{-2}	°C	MJ m^{-2}	°C	MJ m^{-2}	°C	MJ m^{-2}
Oct. (planting)	12.5	15.5	13.4	16.5	14.2	15.7	14.9	17.2
Nov.	17.5	20.5	16.8	18.4	17.0	20.7	17.2	18.5
Dec.	20.9	20.4	19.8	23.9	19.0	20.4	19.7	20.6
Jan. (silking)	19.9	20.9	20.7	21.6	22.3	22.2	20.0	22.8
Feb.	19.1	18.3	19.2	19.9	18.6	19.7	20.7	20.0
Mar. (R6)	17.3	15.3	19.7	16.0	18.0	15.4	18.6	13.3

fected grain yield although symptoms were observed only in less than 10% of the plants. Small yield variations over time could be explained by variation in air temperature and incident radiation (Table 2) because the crop yield (for the highest N rate) was significantly correlated (r^2 = 0.52) with the photothermal coefficient (mean incident radiation/mean air temperature).

Crop Nitrogen Accumulated, Grain Yield, and Nitrogen Recovery

Grain yield was significantly affected by N rate each year and by fertilization time in all but the 1996–1997 season (Table 3). In the 1994–1995, 1995–1996, and 1998–1999 growing seasons, the FV6 increased grain yield compared with FPL, mainly in the lower N rates (Table 3). Greater grain yield with FV6 has been reported by other researchers (Wells and Bitzer, 1984; Fox et al., 1986; Wells et al., 1992).

Grain N accumulation increased by increasing N rate in all growing seasons and with FV6 in 1994–1995, 1995–1996, and 1998–1999 growing seasons. In the 1996–1997 growing season, grain N accumulation was not significantly increased by FV6 compared with FPL (Tables 4 and 5). Nitrogen returned by crop stover increased with increasing N rate in all growing seasons and by FV6 in 1995–1996 and 1998–1999 growing seasons (Tables 4 and 5). Root-returned N behaved similarly to grain N accumulation (Tables 4 and 5), behavior expected because this variable was determined as a proportion of N in the aerial biomass.

The FV6 increased soil NO_3^-–N content at flowering and crop N uptake after that stage with respect to FPL in the 1994–1995, 1995–1996, and 1998–1999 growing seasons (data not shown). In the 1996–1997 growing season, irrigation and rainfall were much greater than CET in January (Fig. 1). Therefore, water excess could have increased N losses from mineral pool, decreasing the difference in crop N uptake after flowering between both fertilization times.

These results indicate that N recovery by the maize crop was greater when N was applied at the V6 stage than when N was applied at planting time. Additionally, it appears that the recovered N was predominately deposited into the grain as previously reported by Bigeriego et al. (1979). The larger rate of N recovery with the FV6, which is just before maximum plant need, may be due to lower N losses through denitrification or leaching or lower N immobilization in organic forms (Bigeriego et al., 1979; Wells and Bitzer, 1984; Jokela and Randall, 1997).

Mean fertilizer N recovery as estimated by the difference method (estimated by subtracting N uptake of the control treatment from the N uptake of the fertilized treatments) ranged from 43 to 53% when the fertilizer was applied at planting time. These values are greater than those reported by Olson (1980) but similar to those

Table 3. Analysis of variance of grain yield of maize for different N rates and application times, planting (P) and V6 stage (V6), in the 1994–1995, 1995–1996, 1996–1997, and 1998–1999 growing seasons.

	Grain yield			
N rate	**1994–1995**	**1995–1996**	**1996–1997**	**1998–1999**
kg ha^{-1}	Mg ha^{-1}			
0	7.2	7.3	6.7	7.8
70 (P)	9.4	9.2	8.8	8.1
140 (P)	11.7	12.9	–	9.3
210 (P)	–	14.0	10.7	10.5
70 (V6)	10.5	11.2	9.6	9.6
140 (V6)	12.0	13.7	–	10.8
210 (V6)	–	13.5	11.0	10.2
CV, %	4.9	6.1	4.7	7.1
	Analysis of variance			
Source of variation				
N	***	***	***	***
Time (T)	*	†	ns	*
N × T	ns	*	ns	†
LSD N‡	0.74	0.97	0.88	0.89
LSD T	0.74	ns	ns	0.72
LSD N × T	ns	1.3	ns	ns

* Difference significant at the 0.05 probability level.

*** Difference significant at the 0.001 probability level.

† Difference significant at the 0.1 probability level.

‡ Least significant difference was calculated only when the main effects or their interaction were significant to 5%.

Table 4. Nitrogen balance in the soil-crop system as affected by N rates and fertilization times, at planting (P) and V6 stage (V6), for the 1994–1995 and 1995–1996 growing seasons.

	Crop available N†		Components of N balance determined‡							
N rate	N_{min}	N_{sinb}	N_{grain}	N_{stover}	N_{root}	MBN	N_{vol}	C_L N_2O-N	N_{sine}	UN§
					1994–1995 Growing season					
					kg ha^{-1}					
0	96.0	75	62	25	5	−3.6	1.3	ND¶	22	59 (11)#
70 (P)	96.0	75	87	35	8	10.3	2.8	ND	15	83 (15)
140 (P)	96.0	75	125	45	11	−0.7	7.6	ND	15	108 (17)
70 (V6)	96.0	75	104	34	9	18	5.0	ND	19	52 (8.6)
140 (V6)	96.0	75	133	55	12	1.7	15.6	ND	32	62 (19)
							Analysis of variance			
Source of variation										
N			**	**	**	ns	**	–	*	*
Time (T)			*	ns	**	ns	*	–	*	**
N × T			ns	ns	ns	ns	ns	–	*	ns
LSD N††			9	6	0.5	ns	4.5	–	–	22
LSD T			7	ns	0.4	ns	2.5	–	–	22
LSD N × T			ns	ns	ns	ns	ns	–	10	ns
					1995–1996 Growing season					
					kg ha^{-1}					
0	118	72	56	30	6	24	0.3	ND	29	45 (7)
70 (P)	118	72	77	33	6	44	2.0	ND	29	69 (10)
140 (P)	118	72	128	47	9	39	9.0	ND	28	70 (19)
210 (P)	118	72	159	55	11	26	17.4	ND	31	101 (14)
70 (V6)	118	72	110	39	8	29	6.4	ND	33	35 (15)
140 (V6)	118	72	150	56	11	33	16.5	ND	35	29 (12)
210 (V6)	118	72	171	61	12	53	28.3	ND	68	7 (5)
							Analysis of variance			
Source of variation										
N			**	**	**	*	**	–	*	ns
Time (T)			**	*	**	ns	*	–	*	**
N × T			ns	ns	ns	*	ns	–	*	*
LSD N			10	9	1.0	10.2	5.9	–	–	ns
LSD T			7	6	0.7	–	4.2	–	–	17.0
LSD N × T			ns	ns	ns	19.2	ns	–	23	25.0

* Difference significant at the 0.05 probability level.

** Difference significant at the 0.01 probability level.

† N_{min}, mineralized N during the growing season; N_{sinb}, inorganic N at the beginning of the growing season (0–100 cm).

‡ N_{grain}, N content in grain; N_{stover}, N content in stover; N_{root}, N content in root system; MBN, change in soil microbial biomass N content (0–20 cm soil depth) between physiological maturity (end) and planting (beg), i.e., MBN = $MBN_{end} - MBN_{beg}$; N_{vol}, volatilization losses; C_L N_2O-N, cumulative N_2O-N losses; N_{sine}, inorganic N at the end of the growing season (0–100 cm).

§ UN, unaccounted N.

¶ ND, not determined.

\# Values in parentheses represent standard deviation.

†† Least significant difference was calculated only when the main effects or their interaction were significant to 5%.

reported by other authors (Kitur et al., 1984; Reddy and Reddy, 1993; Jokela and Randall, 1997). The FV6 resulted in estimated fertilizer N recovery values between 62 and 74%, which is less than those reported by Bigeriego et al. (1979) but greater than those reported by Jokela and Randall (1997). The difference with these last authors can be explained by greater water availability in our experiment after the V6 stage. On the other hand, when N recovery was calculated by the whole-crop available N method (mineralized N plus mineral N at planting plus N fertilizer), the observed values were 46 and 53% for FPL and FV6, respectively (Tables 4 and 5).

Nitrogen in Soil Microbial Biomass

Nitrogen immobilization by microbial biomass was increased by N fertilization in all growing seasons but the 1994–1995 season (Tables 4 and 5). The lack of N immobilization determined in 1994–1995 with respect to the other growing seasons could be explained by the effect of C availability on soil biomass size (Bonde et al., 1987) because the preceding crop was soybean in 1994–1995 and maize in other growing seasons. In turn, N immobilization was not different for the fertilized treatments, and the main difference was observed between control and the fertilized treatments (Tables 4 and 5). For maize crops growing under rainfed conditions, Jokela and Randall (1997) reported more N in organic forms for FPL compared with FV6. This behavior could be due to longer time and sometimes better moisture conditions for microbial immobilization between the time of application and maximum crop N uptake. However, under irrigation, soil water content after V6 would not be a very restrictive factor of the microbial activity. In Balcarce, mean soil temperature at a 20-cm depth is 14.5 and 17.5°C for October and November (period between planting and V6), respectively (Della Magiora, personal communication, 1985). Therefore, this variable could also have limited microbial growth and N immobilization for planting with respect to V6 fertilization because optimum temperature for soil N mineralization and immobilization ranged from 25 to 30°C (Alexander, 1977).

Microbial biomass organic N transforms progressively

Table 5. Nitrogen balance in the soil–crop system as affected by N rates and fertilization times, at planting (P) and V6 stage (V6), for the 1996–1997 and 1998–1999 growing seasons.

	Crop available N†		Components of N balance determined‡							
N rate	**N_{min}**	**N_{sinb}**	**N_{grain}**	**N_{stover}**	**N_{root}**	**MBN**	**N_{vol}**	**C_L N_2O-N**	**N_{sine}**	**UN§**
					1996–1997 growing season					
					kg ha^{-1}					
0	**145**	**53**	**48**	**44**	**6**	**12.3**	**0.0**	**3.8**	**19**	**65 (7)¶**
70 (P)	**145**	**53**	**79**	**57**	**7**	**31.3**	**0.0**	**7.4**	**25**	**61 (24)**
210 (P)	**145**	**53**	**123**	**85**	**11**	**41.7**	**0.0**	**10.4**	**22**	**115 (21)**
70 (V6)	**145**	**53**	**86**	**55**	**7**	**18.0**	**ND#**	**4.5**	**31**	**66 (19)**
210 (V6)	**145**	**53**	**132**	**90**	**12**	**28.7**	**ND**	**5.4**	**39**	**99 (21)**
							Analysis of variance			
Source of variation										
(N)			**	***	***	*	–	***	**ns**	*
Time (T)			**ns**	**ns**	**ns**	**ns**	–	***	*	**ns**
N × T			**ns**	**ns**	**ns**	**ns**	–	**	**ns**	**ns**
LSD N††			**12**	**9**	**0.9**	**13.6**	–	**0.8**	**ns**	**25.2**
LSD T			**ns**	**ns**	**ns**	**ns**		**0.8**	**7.4**	**ns**
LSD N × T			**ns**	**ns**	**ns**	**ns**		**1.2**	**ns**	**ns**
					1998–1999 Growing season					
					kg ha^{-1}					
0	**132**	**88**	**62**	**30**	**6**	**28.0**	**ND**	**7.1**	**25**	**62 (18)**
70 (P)	**132**	**88**	**81**	**29**	**6**	**33.7**	**ND**	**11.9**	**41**	**87 (25)**
140 (P)	**132**	**88**	**96**	**45**	**7**	**43.3**	**ND**	**14.9**	**42**	**112 (21)**
210 (P)	**132**	**88**	**116**	**51**	**9**	**33.3**	**ND**	**15.0**	**41**	**163 (18)**
70 (V6)	**132**	**88**	**92**	**42**	**7**	**38.0**	**0.0**	**8.3**	**46**	**55 (32)**
140 (V6)	**132**	**88**	**119**	**49**	**9**	**35.0**	**0.0**	**7.7**	**60**	**71 (13)**
210 (V6)	**132**	**88**	**127**	**75**	**11**	**41.0**	**0.0**	**7.2**	**88**	**80 (27)**
							Analysis of variance			
Source of variation										
N			***	***	***	‡‡	–	***	**	***
Time (T)			*	**	***	**ns**	–	***	***	***
N × T			**ns**	*	**ns**	**ns**	–	*	**	*
LSD N			**13**	**7**	**0.9**	**ns**	–	**2.2**	**9.0**	**8.8**
LSD T			**9**	**5**	**0.6**	**ns**	–	**2.2**	**7.3**	**6.2**
LSD N × T			**ns**	**10**	**ns**	**ns**	–	**2.8**	**13.1**	**18.5**

*** Difference significant at the 0.05 probability level.**
**** Difference significant at the 0.01 probability level.**
***** Difference significant at the 0.001 probability level.**
† N_{min}, mineralized N during the growing season; N_{sinb}, inorganic N at the beginning of the growing season (0–100 cm).
‡ N_{grain}, N content in grain; N_{stover}, N content in stover; N_{root}, N content in root system; MBN, change in soil microbial biomass N content (0–20 cm soil depth) between physiological maturity (end) and planting (beg), i.e., MBN = $MBN_{end} - MBN_{beg}$; N_{vol}, volatilization losses; C_L N_2O-N, cumulative N_2O-N losses; N_{sine}, inorganic N at the end of the growing season (0–100 cm).
§ UN, unaccounted N.
¶ Values in parentheses represent standard deviation.
 # ND, not determined.
†† Least significant difference was calculated only when the main effects or their interaction were significant to 5%.
‡‡ Difference significant at the 0.1 probability level.

into more stable organic compounds as the microbial biomass dies (Jenkinson and Parry, 1989). Data from the Broadbalk experiment show that the half-life for soil microbial biomass is on the order of 1 yr (Jenkinson and Parry, 1989). Moreover, these authors suggest that a large proportion of the organic products liberated by dead microbial cells are mineralized and again used for the growth of new microbial populations (cryptic growth). These antecedents would indicate that in our experiment, N immobilization would not have been largely underestimated if we had only considered the MBN since a great part of the N immobilized from the fertilizer would be part of this N pool at the end of the growing season. For the 1995–1996, 1996–1997, and 1998–1999 growing seasons, the mean value for the immobilized N fertilizer was estimated to be 13.4 kg N ha^{-1} and was calculated as the difference between fertilized treatments (average N rates) and the control treatment. This value is lower than that reported by Olson (1980), but similar to that of Reddy and Reddy (1993), for fertilizations at planting time for maize grown under CT management.

In the 1995–1996 and 1996–1997 growing seasons, no significant relationship was found between crop N uptake and change in MBN (MBN at physiological maturity minus MBN at planting) in the preceding growing season (Fig. 2a). Therefore, this N pool would not have contributed to N available for these treatments. This result can be explained by the low N immobilization determined in each growing season (Tables 4 and 5) and by the progressive transformation, as the microbial biomass dies, into active humus N, which has a turnover time of 3.1 yr (Jenkinson and Parry, 1989). Therefore, the change in MBN in the preceding growing season would not have contributed to available N, and therefore the UN for the fertilized treatments would not have been largely overestimated.

Ammonia and Denitrification Losses

The dynamics and the magnitude of ammonia N losses from the urea were discussed in a previous paper (Sainz

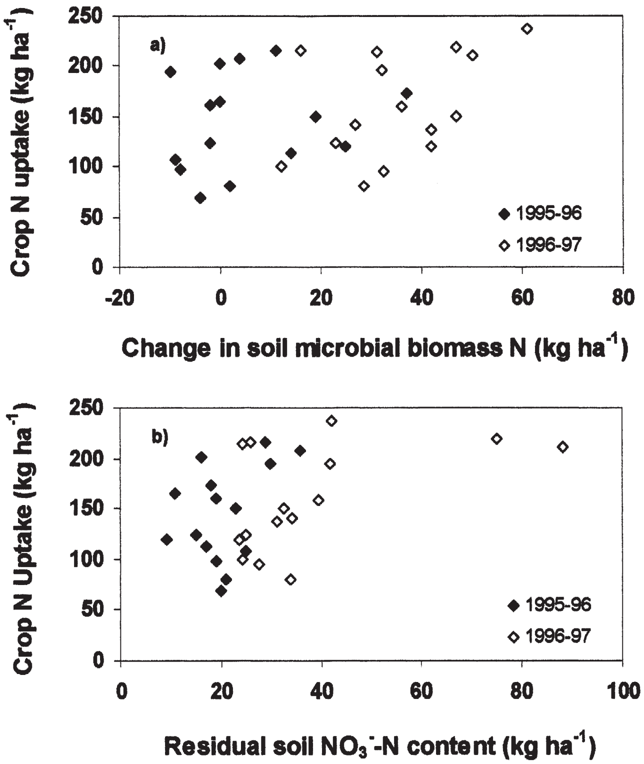

Fig. 2. **Crop N uptake for unfertilized and fertilized treatments in the 1995–1996 and 1996–1997 growing seasons in relation to (a) the change in soil microbial biomass N for the period between planting and physiological maturity of the preceding season (1994–1995 or 1995–1996) and (b) residual NO_3^-–N measured at the physiological maturity growth stage of the preceding season.**

Rozas et al., 1999). Ammonia N losses increased with N rate, and they were greater for FV6 fertilization than for FPL (Table 4). Greater losses observed for FV6 were highly associated with higher soil temperatures at that time (Sainz Rozas et al., 1997b). Denitrification N rates and their relation with soil factors were also discussed in a previous paper (Sainz Rozas et al., 2001). Cumulative N_2O-N losses (C_L N_2O-N) showed in this paper are greater than those reported by these last authors because denitrification rates were accumulated from planting to flowering in the 1996–1997 growing season and from planting to R6 in 1998–1999 growing season. Nevertheless, the results follow the same pattern as those reported by Sainz Rozas et al. (2001). A significant interaction was observed between N rate and fertilization time for C_L N_2O-N because N rate increased C_L N_2O-N only when N was applied at planting time (Table 5). Despite the interaction, in both growing seasons, C_L N_2O-N were significantly greater for FPL than FV6 (Table 5). In the 1996–1997 growing season, C_L N_2O-N as a percentage of applied N for FPL were 5.1 and 3.1% for N rates of 70 and 210 kg N ha^{-1}, respectively. In 1998–1999, C_L N_2O-N were 6.8 and 3.8% for N rates of 70 and 210 kg ha^{-1}, respectively (Table 5). However, in both growing seasons, when the urea was applied at the V6 stage, C_L N_2O-N ranged between 0.05 and 1.71% of applied N (Table 5). The relatively low N_2O-N losses can be explained by the amount of C susceptible to mineralization under anaerobic conditions (Sainz Rozas et al., 2001).

Residual Soil Nitrate

In the 1994–1995, 1995–1996, and 1998–1999 growing seasons, soil inorganic N at the end of the growing season was significantly affected by an interaction between N rate and fertilization time. Mineral N was significantly greater only for the highest N rate applied to V6 stage (Tables 4 and 5), and it indicates that high N rates applied at that stage exceeded crop requirements. Similar results have been reported by Bigeriego et al. (1979) and Jokela and Randall (1997). When N remaining in the soil profile after maize harvest is high, the potential for NO_3^-–N pollution of groundwater increases significantly from harvest to the next spring (Liang et al., 1991). However, for FPL, N rate did not increase soil NO_3^-–N content at the end of the growing season. This result would indicate that greater N losses, maybe through nitrate leaching, happened during the growing season for this fertilization time.

In the 1995–1996 and 1996–1997 growing seasons, no significant relationship was found between crop N uptake for unfertilized and fertilized treatments and residual NO_3^-–N in the preceding growing season (Fig. 2b), and therefore, this N pool would not have contributed to available N, and the UN for the fertilized treatments would not have been largely overestimated. This result can be explained by the low soil NO_3^-–N content determined at physiological maturity in the 1994–1995 and 1995–1996 growing seasons (Tables 4 and 5) and by N losses happening during fallow period.

Unaccounted Nitrogen

In 1995–1996 and 1998–1999, there was a significant interaction ($P < 0.05$) between N rate and the fertilization time for the UN. Increasing N rate increased UN mainly for FPL (Tables 4 and 5). Despite the interaction, the UN for FPL was significantly higher than FV6 in the 1994–1995, 1995–1996, and 1998–1999 growing seasons (Tables 4 and 5). In the 1996–1997 growing season, UN was affected only by the N rate (Table 5). As it has been mentioned, water excess in January (Fig. 1) could have caused nitrate leaching, diminishing the difference between fertilization times.

Air mean temperatures of October and December (time of planting and V6 fertilization, respectively) were similar in the 1994–1995 and 1995–1996 growing seasons (Table 1). Consequently, volatilization losses for the same N rate and application time were similar (Table 4) because volatilization rate and air mean temperature are highly associated (Sainz Rozas et al., 1997b). Therefore, volatilization losses would not have been very different in the other years in which these losses were not determined, because air mean temperature of October and December showed little variation through the years (Table 1).

For both fertilization times, almost all denitrification losses were measured between planting and flowering (period elapsed between October and January) because after flowering, denitrification rate is limited by N availability (Sainz Rozas et al., 2001). Accumulated rainfall plus irrigation between October and January ranged from 523 to 403 mm for 1996–1997 and 1998–1999 growing seasons, respectively (Fig. 1). Nevertheless, C_L N_2O-N, as a percentage of applied N, were something higher in 1998–1999 than 1996–1997 growing season (Table 5). Therefore, these losses would not have been very different in the others years because accumulated rainfall plus irrigation between October and January ranged from 474 to 521 mm for 1994–1995 and 1995–1996 growing season, respectively (Fig. 1).

Unaccounted N in 1994–1995 and 1995–1996 for both application times, and for FV6 and FPL in 1996–1997 and 1998–1999 growing seasons, respectively, cannot be attributed solely to NO_3^-–N leaching losses. This is because denitrification losses were not determined in the first two growing seasons and volatilization losses were not determined in the last two growing seasons. For FPL, when denitrification or volatilization losses (average of years) were subtracted from UN in the years in which these losses were not determined, the mean UN (through the years) was 55, 69, 86, and 103 kg N ha^{-1} for 0, 70, 140, and 210 kg N ha^{-1}, respectively. These values represent N losses of 20.0, 22.1, and 22.8% of applied N. However, for FV6, these values were 56, 46, 49, and 34 kg N ha^{-1} for the same N rates. The lower N losses for the fertilized treatments at V6 with respect to the control treatment are explained by an overestimation of different N pools determined. Greater crop N uptake from soil organic matter and increased root growth in fertilized plots (Rao et al., 1991) could partially explain the overestimation and the negative values observed for FV6. For FPL, this effect would have been counteracted by the existence of greater N losses from soil. However, the overestimation for the treatments fertilized at V6 was not very important.

Unaccounted N for FPL could be attributed to NO_3^- leaching because rainfall was higher than CET at the beginning of the growing season while for FV6, early December, rainfall plus irrigation generally were less or slightly higher than CET after the FV6 (Fig. 1). From experiments with continuous maize crops fertilized at planting time with 200 kg N ha^{-1}, Toth and Fox (1998) reported that rainfall events greater than 15 mm immediately after surface fertilization increased the leachate NO_3^-–N concentration early in the growing season and increased NO_3^- leaching. These results are in agreement with those observed in our experiment due to rainfall events that happened from 1 to 8 d after fertilization.

Nitrate leaching losses determined indirectly in this experiment for the two higher N rates applied at planting time are lower than nitrate leaching loss reported by Walters and Malzer (1990). These authors worked with maize under CT in sandy soils and reported NO_3^- leaching loss of 30% of N applied (180 kg N ha^{-1}). However, the NO_3^-–N leaching loss in our study is higher than losses reported by Jeminson et al. (1994). These authors worked on silt-loam texture soils and reported NO_3^- leaching losses of 14% of N applied (200 kg N ha^{-1}) for the same application time under CT. These losses happened mainly at the beginning of the growing season. This difference would be attributed to the larger infiltration capacity in soils under NT than CT because of the presence of surface mulch and the larger number of continuous macropores that are open at the soil surface (Unger and McCalla, 1980). At Balcarce, in a maize crop under CT fertilized at planting time with 200 kg N ha^{-1}, Costa et al. (2003) reported leaching losses of 66.5 kg N ha^{-1}. These losses could be higher than those reported in our experiment because these authors evaluated leaching losses for a longer period.

Nitrogen Fate as Affected by Rate and Application Time

When N fertilizer was applied at the V6 stage, the crop accumulated a greater proportion of the applied fertilizer N than when the fertilizer was applied at planting time (Fig. 3). Total gaseous losses (denitrification plus volatilization) represented a fraction ranging from 7.6 to 13.8% of applied N (Fig. 3). Denitrification losses were more important for FPL and accounted for almost 80% of total gaseous losses for the lowest N rate (70 kg N ha^{-1}) while volatilization was a more important mechanism loss for FV6 and accounted for up to 82% of total gaseous losses for the highest N rate (210 kg N ha^{-1}) (Fig. 3).

Leached NO_3^-–N represented a fraction of the applied N ranging from 20 to 22.8% when this nutrient was applied at planting (Fig. 3). Total gaseous losses plus NO_3^- leaching represent a fraction ranging from 28.9 to 34% of fertilizer N (Fig. 3), or a fractional recovery within the entire soil–crop system ranging from 66 to 71%. These values are similar to those reported by Meisinger (1984). However, for FV6, the fractional recovery within the entire soil–crop system ranged from 96 to 104% of applied N.

Residual NO_3^-–N increased with increasing N rate for FV6, but this N pool did not increase for FPL (Fig. 3). Therefore, it is very important that producers do not apply N in excess of crop requirements because N remaining in the soil profile after harvest is susceptible to leaching and thus increases the risk of groundwater pollution.

The MBN was similar for both fertilization times (Fig. 3), and therefore, the greater N uptake and grain yield for the V6 fertilization were attributed to the existence of greater N leaching for the planting fertilization because total gaseous losses were similar for both fertilization times.

CONCLUSIONS

Results of this study indicate that NO_3^-–N leaching would be an important N loss mechanism operating in NT maize cropping systems in the southeastern of Buenos Aires province. However, grain yield, N uptake, and NUE were increased when N was applied just before maximum plant need since total gaseous losses are low and N leaching is reduced, aspects important from economic and environmental standpoints.

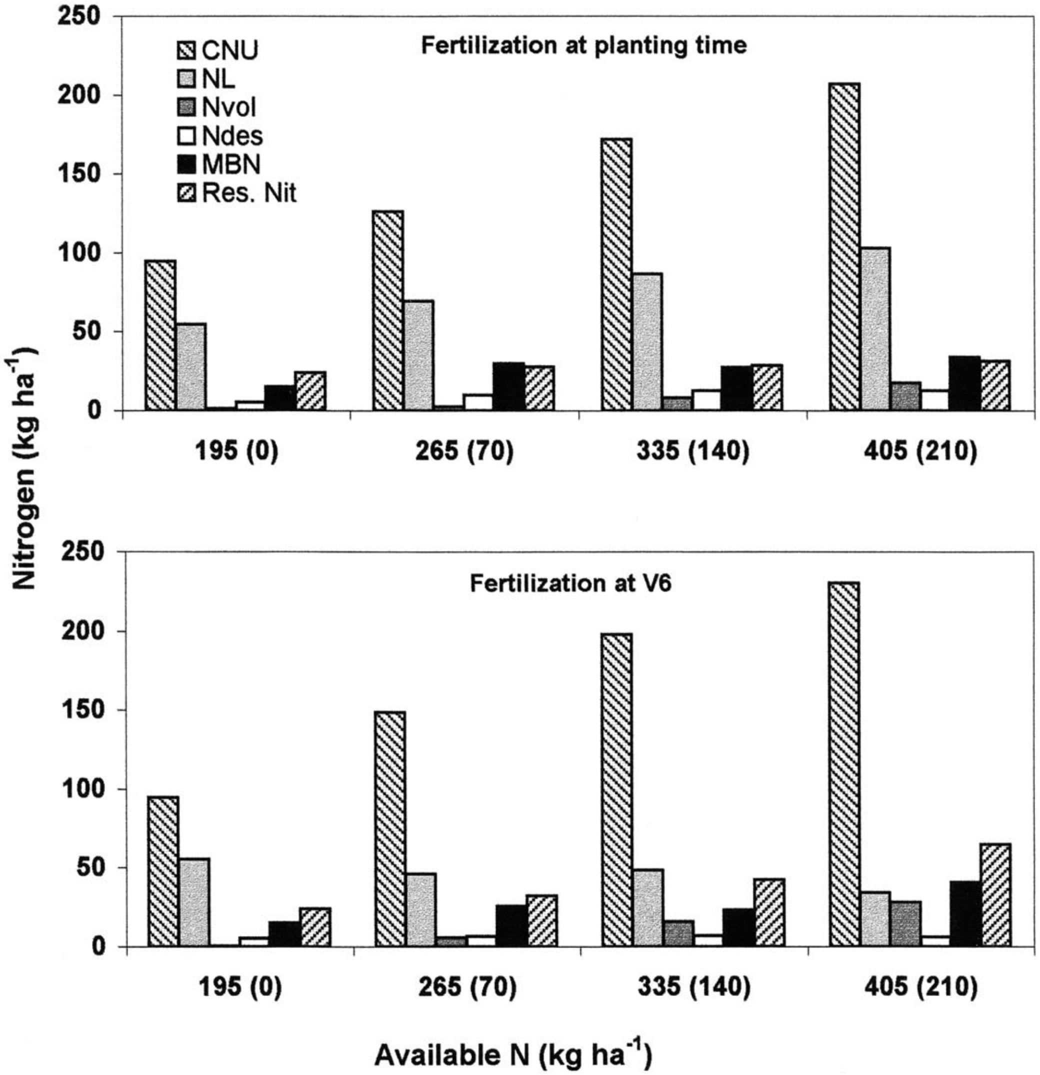

Fig. 3. Components of N balance as a function of different N rates and application times. Available N represents soil NO_3^-–N at planting time (0- to 100-cm soil depth) plus N applied (N rates are shown in parentheses) and mineralized N from organic matter. Res. Nit = soil NO_3^-–N at physiological maturity (0- to 100-cm soil depth); MBN = change in soil microbial biomass N content (0- to 20-cm soil depth) between physiological maturity (end) and planting (beg), i.e., MBN = $MBN_{end} - MBN_{beg}$; Ndes = denitrification loss; Nvol = volatilization loss; NL = apparent N loss by leaching; and CNU = N accumulated by the crop.

ACKNOWLEDGMENTS

This work was supported by the project AGR 163/03 of the FCA-UNMP and by resources of the Agricultural Experimental Station INTA of Balcarce.

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