ISSN: 0190-4167 print / 1532-4087 online DOI: 10.1080/01904160802714961



# Effect of Water Stress in Maize Crop Production and Nitrogen Fertilizer Fate

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## **ABSTRACT**

Maize production is affected by water and nitrogen (N) deficit either separately or joined, but this fact is not completely defined. The aim was to evaluate the fate of N in maize fertilized and subjected to water stress in controlled conditions. A greenhouse experiment was carried out at the University of Buenos Aires campus. The design was a 2  $\times$  2 factorial with four replications. The factors were N: 70 and 140 kg N ha<sup>-1</sup> as labeled urea (15N), and water: 100% or 50% of the potential evapotranspiration. The harvest of aerial and root biomass was carried out at R1 stage. Nitrogen in plants, soils nitrate, ammonia volatilization, and <sup>15</sup>N percentage were determined. Obtained results only partially agree with previous research. Water stress depressed aerial biomass production independently of N doses. When water was limiting, the uptake of N from fertilizer was independent of N. When water was not limiting, N uptake increased with the higher N doses. Volatilization losses were 3.7 to 7.8% of N applied as fertilizer. Plant N recovers was around 45% of the N applied, except in treatment water stressed with high N rate (19%). Nitrate-N from the fertilizer in the soil at harvest and accumulated N from the fertilizer in plant were lineally related ( $r^2 = 0.54$ ; p < 0.001). Important destinations of N were accumulation in plant, volatilization and incorporation into soil organic matter. However, residual nitrate was a main fate in heavily fertilized and water deficit treatment. This process could lead to the eventual nitrate leaching.

**Keywords:** nitrogen recovery, 15N, labeled nitrogen, maize, water stress, fertilizer fate, nitrate leaching, ammonia volatilization

Received 19 October 2007; accepted 27 December 2008.

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## INTRODUCTION

Water deficit has been identified as the single most yield-reducing factor for crops (Bohnert and Bressan, 2001). Periods of water deficit may occur frequently even in regions characterized by high annual rainfall. Water deficit affects growth, and decreases the conversion of radiation into biomass in maize (*Zea mays* L.) (Otegui et al., 1995). The most sensitive period of maize to drought is between 2 weeks before and 2–3 weeks after silking (Hall et al., 1992; Otegui et al., 1995). Water deficit may also affect below-ground biomass accumulation growth. Sharp and Davies (1979) found that mild water stress may initially increase root growth, but severe and long lasting drought decreases root growth (Eghball and Maranville, 1993).

Nitrogen (N) deficiency is other major factor controlling maize yields worldwide (Alvarez and Grigera, 2005). Nitrogen stress decreases the leaf expansion and photosynthetic rate (Novoa and Loomis, 1981; Muchow, 1989). Nitrogen deficiency also decreases maize yield due to lower grain number and weight. This is caused by fewer fertilized ovum, kernel abortion and other changes at the physiological and biochemical level (Uhart and Andrade, 1995).

The effect of the combined effect of water and N deficits on maize growth has been intensively studied recently (Rees et al., 1996; Sainz Rozas et al., 2004). However, we are still far from having a comprehensive understanding of this interaction. When maize was subjected to drought, Pandey et al. (2000a) and Moser et al. (2006) found a lower yield with high N doses. However, Eck (1984) and Al-Kaisi and Yin (2003) observed that high N doses do not affect yield in water stressed plants. At the below-ground level, Eghball and Maranville (1993) established that root biomass was not affected by the interactive effect of N rate and water deficit. Those uneven results reflect the fact that drought treatments are difficult to standardize due to each local environmental characteristics, soil properties and crop attributes. Drought effect also depends on its absolute value and its temporal variation (Buljovcic and Engels, 2001; Panda et al., 2004).

When the yield is drought-limited the proportion of N from fertilizers recovered by crops is usually low, although it can vary according management practices (Ma et al., 1995; Macdonald et al., 1997; Hood et al., 1999). Non-recovered N can enter into the soil organic fraction, or lost by leaching, volatilization, denitrification and other ways. To quantify the proportion of N from fertilizer taken by the crops or following other destination <sup>15</sup>N isotope can be used (Ma et al., 1995; Gorfu et al., 2003; Herzog and Götz, 2004). This is a direct method to determine the fate of N from fertilizer through the soil and plant compartments and through the air and water (Carter and Rennie, 1987). Using <sup>15</sup>N technique, Macdonald et al. (1997) found that the recovery of N from fertilizer varied from 26 to 60%, depending on the crop and environmental conditions. When crop yield was limited by unfavorable climatic conditions or diseases, the percentage of N recovered by plants decreased and a significant proportion of labeled N was found in soil depth (Macdonald et al., 1997).

The objective was to study the fate of N from fertilizer applied to maize plants subjected to water stress in controlled conditions.

## MATERIALS AND METHODS

An experiment was carried out in a greenhouse located at the College of Agronomy, University of Buenos Aires, Buenos Aires, Argentina (34° 36′S, 58° 29′W). Maize seeds (Cargill Titanium F1) were pre-germinated for 4 days at 25°C and then transplanted (November 15th, 2001) into 50 cm diameter and 60 cm deep plastic containers. The substrate used was a mix of soil (topsoil of a Typic Argiudoll) and sand (3:1 ratio), to maintain good physical conditions in the containers. The main physical and chemical characteristics of that substrate are shown in Table 1. Determinations were carried out using standard analytical methods (Sparks et al., 1996). All containers receive 2 g of triple superphosphate (equivalent to 20 kg P ha<sup>-1</sup>). Insects were chemically controlled.

The design of the experiment was a  $2 \times 2$  factorial (nitrogen  $\times$  water) with four replications for each treatment. Nitrogen factor included two levels: N70 (equivalent to 70 kg N ha<sup>-1</sup> and N140 (equivalent to 140 kg N ha<sup>-1</sup>). Fertilization was performed using labeled urea with <sup>15</sup>N (1.5% abundance) when plants had six leaves, V6 stage (Ritchie and Hanway, 1982). Urea was banded and semi-incorporated (at around 3 cm depth), representing field conditions.

Water factor included two levels: +water and -water. The +water treatment covered 100% of the estimated potential evapotranspiration, according to Penman method. It was obtained applying 15 mm each three days, totalizing 390 mm during the study period. In this case, the soil water content was maintained most days around 90–100% of the field capacity. The -water treatment covered around 50% of the estimated potential evapotranspiration. It was obtained applying 8 mm every three days, totalizing 200 mm. In this treatment, the soil water content was maintained in an average of 63% of the field capacity.

Table 1
Substrate characteristics

Substrate characteristics	Level	
Oxidable Carbon (g kg <sup>-1</sup> )		
pH	6.0	
Available Phosphorus (mg kg <sup>-1</sup> )	13.2	
Nitrates-N (mg kg <sup>-1</sup> )	1.8	
Clay $(g kg^{-1})$	236	
Silt $(g kg^{-1})$	216	
Sand $(g kg^{-1})$	547	

Aerial biomass and roots were harvested 77 days after planting, when plants were at R1 stage (stigmas emergence).

# **Analytical Procedures**

Plant material was dried at 60°C and Total N by Kjeldahl method (Bremner and Mulvaney, 1982) and <sup>15</sup>N percentage, using optical emission spectrometry (Fiedler and Proksch, 1975) were determined.

Nitrate concentration in soil was determined at the end of the experiment. Soil samples were taken from the containers to a 60 cm depth. Nitrate concentration was determined by the Devarda alloy and airstreams distillation method (Sparks et al., 1996) and  $^{15}$ N percentage with the technique above described. Nitrate in soil mass were calculated using nitrate concentration and bulk density (1.1 g cm $^{-3}$ ).

Total volatilization losses were determined using the chamber method proposed by Nommik (1973), during 50 days from fertilization date. Ammonia was trapped with sulfuric acid-glycerol solution, subjected to airstream distillation, and determined using a colorimeter (Sparks et al., 1996). The <sup>15</sup>N percentage was determined as above described. One chamber was placed over the fertilization band and the other far from it. It was assumed that the ammonia volatilized from the fertilization band represent 32% of the total area of the container. The other chamber represented the remaining 68% of that area. Volatilization losses for each treatment were expressed as g ammonia (NH<sub>3</sub>)-N m<sup>-2</sup> accumulated in the period.

Results were analyzed using ANOVA. Contrasts between treatments were tested with Student's t-test. Relations between variables were tested applying simple regression.

# **Calculations**

The percentage of N derived from fertilizer (NdfF) in each compartment was calculated using equation 1. The natural abundance of <sup>15</sup>N was considered as 0.366%.

NdfF (% ) = (% atom 
$$^{15}$$
N in soil, plant or gas samples 
$$-\% \, ^{15}$$
N natural abundance)/(% atom  $^{15}$ N in fertilizer 
$$-\% \, ^{15}$$
N natural abundance) \* 100 (1)

The fertilizer plant recovery was calculated using equation 2:

Fertilizer plant recovery (%) = NdfF 
$$_{plant}/N_{fertilization} * 100$$
 (2)

Where  $NdfF_{plant} = Nitrogen$  in plant derived from fertilizer (g N plant<sup>-1</sup>);  $N_{fertilization} = Nitrogen$  applied by fertilization (g N pot<sup>-1</sup>).

The fate of N applied as fertilizer was calculated using equation 3:

$$\begin{split} N_{\text{fertilizer}}\left(100\%\right) &= \text{NdfF}_{\text{plant}}\left(\%\right) + \text{NdfF}_{\text{volatilized}}\left(\%\right) \\ &+ \text{NdfF}_{\text{nitrate}}\left(\%\right) + \text{NdfF}_{\text{unaccounted}}\left(\%\right) \end{split} \tag{3}$$

Where  $N_{fertilizer}$  = Fertilizer applied N (100%); NdfF<sub>plant</sub> = N from fertilizer taken by the whole plants (aerial +root biomass) (%); NdfF<sub>volatilized</sub> = N volatilized from fertilizer (%); NdfF<sub>nitrate</sub> = N from fertilizer remaining in the soil as nitrate (%); NdfF<sub>unaccounted</sub> = N from fertilizer located in non measured sinks (%).

## RESULTS AND DISCUSSION

#### **Biomass Production**

Maize without water restrictions (+water treatment) produced higher total biomass (aerial + root biomass) than plants suffering water stress (—water treatment) (p < 0.0001). There were not significant differences between N doses (p = 0.2849). Aerial and root compartments were affected in different way by the N fertilization and water regime (Tables 2 and 3). Water stress depressed aerial biomass production independently of N fertilization doses (non significant interaction N \* drought). This result agree with those of Eck (1984) and Al-Kaisi and Yin (2003). Maize roots showed a different pattern of response

Table 2

Dry matter, nitrogen concentration and absorbed in maize. Standard error is given between parentheses

	Dry matter (g plant <sup>-1</sup> )	N concentration (g N per kg plant <sup>-1</sup> )	N absorbed (g N plant <sup>-1</sup> )
Above-ground			
N70 —water	146.91 (13.35)	13.12 (0.57)	1.93 (0.11)
N140 -water	152.72 (6.67)	12.45 (0.29)	1.90 (0.09)
N70 +water	260.86 (3.24)	8.13 (0.39)	2.12 (0.14)
N140 + water	266.59 (19.97)	9.67 (0.24)	2.58 (0.26)
Below-ground			
N70 -water	10.98 (2.08)	11.05 (0.93)	0.12 (0.03)
N140 -water	21.55 (2.63)	13.92 (2.21)	0.30 (0.03)
N70 + water	24.21 (2.11)	8.50 (0.93)	0.21 (0.04)
N140 +water	29.13 (2.71)	13.28 (2.99)	0.39 (0.09)

Table 3
ANOVA p values for the effect of water (W) and nitrogen (N) availability

	Main factors		
Trait	W	N	$\begin{aligned} & \text{Interaction} \\ & W \times N \end{aligned}$
		p	
Above-ground			
Dry matter	< 0.001	0.619	0.997
N concentration	< 0.001	0.289	0.018
Total N absorbed	0.007	0.127	0.110
N absorbed from fertilizer	0.028	0.043	0.010
N absorbed from soil	0.012	0.463	0.755
Below-ground			
Dry matter	0.001	0.007	0.261
N concentration	0.435	0.077	0.639
Total N absorbed	0.076	0.003	0.954
N absorbed from fertilizer	0.272	0.253	0.510
N absorbed from soil	0.111	0.002	0.700
Fertilizer recovery in plant	0.277	0.019	0.019
Soil residual nitrate			
NO <sub>3</sub> -N from fertilizer	0.021	0.002	0.020
NO <sub>3</sub> -N from soil	0.860	0.135	0.431
Volatilization			
NH <sub>3</sub> -N from fertilizer	0.146	0.016	0.012
NH <sub>3</sub> -N from soil	0.248	0.044	0.087

to the treatments compared to the aerial compartment: both factors, water and N, affected the root but the interaction between them was non significant (Table 3). The lower soil water content and the lower N doses lead to the lower root biomass production. It is well known that the response of the belowground compartments to the stress of water and nutrients depends greatly on the intensity of the deficit. As a consequence, the reports on root response to water stress are far to be uniform. For some authors root length and biomass increased under water stress (Prystupa and Lemcoff, 1998). For others root biomass can be restricted or enhance according drought stress intensity (Sharp and Davies, 1979; Eghball and Maranville, 1993). On the other hand, Eghball and Maranville (1993) observed that root biomass was not affected by the interaction of N rate and water regime. Different to present results, they found that root weight decreased with N application.

# **Nitrogen Concentration**

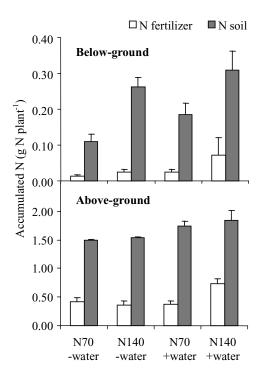
Aerial biomass N concentration showed a significant interaction between water deficit and fertilization (Tables 2 and 3). Maize plants subjected to –water

treatment had similar N concentration, independently of the fertilization treatment. Conversely, the maize plants subjected to the +water treatment showed the higher N concentration under N140 treatment. The maize subjected to +water treatment, however, showed lower N concentration than that on -water treatment. This fact reflects a dilution effect, caused by the higher effect of the N140 treatment on biomass than on N accumulation. Nitrogen concentration in root biomass was not affected by water treatments but on N140 treatment there was a tendency (p = 0.07) to increase root N concentration.

# Nitrogen Uptake

Maize N uptake (aerial + root biomass) was significantly higher in plants subjected to +water and N140 treatments (p = 0.0113). The interaction between both studied factors was not significant.

Water regime affected total N absorption in aerial biomass, independently of the N level (Tables 2 and 3; Figure 1). The higher N accumulation in aerial



*Figure 1.* Accumulated nitrogen in below-ground and above-ground biomass coming from the soil and from the fertilizers. N soil: nitrogen accumulated from the soil; N fertilizer: nitrogen accumulated from fertilizer. Bars = standard error.

biomass was found in the +water treatment, especially in the N140 treatment. The aerial biomass increased was the main cause for the higher N accumulation, since N concentration decreased due to the dilution effect. Al-Kaisi and Yin (2003) also reported that N absorption before different water conditions and fertilization doses was mainly related to biomass increases. N absorbed from fertilizer showed an interaction between both factors (N and W). When water was limiting, the uptake of N from fertilizer was not affected by N doses. On the contrary, when water was not a limiting factor, N uptake increased with the higher N doses. Present data do not agree with results from Pandey et al. (2000b). They found that N absorption by maize depended more from fertilization doses than water restriction.

The absorption of N coming from the soil was higher in plants not subjected to water deficit, independently of the fertilization treatment. It can be attributed to a higher root growth in the +water treatments (Table 2) in agreement with observation from Harmsen and Moraghan (1988).

Fertilization increased the uptake of soil N in the roots, independently from water treatment (Table 3; Figure 1). In this compartment there were no differences in absorbed N from fertilizer. Nitrogen accumulation in plants in a field experiment carried out nearby and in the same year, was within the same order of magnitude than this greenhouse experiment (Rimski-Korsakov et al., 2007).

Plants recovered around half of the applied N fertilizer. The only exception was the –water/N140 treatment, in which only 20% of the fertilizer was recovered by the plants (Figure 2). This is in agreement with findings of Eck

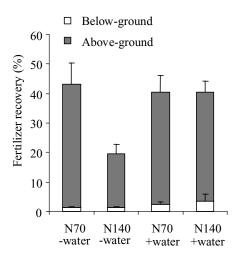
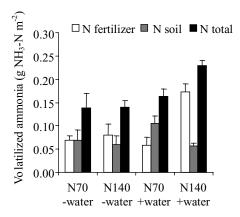


Figure 2. Fertilizer recovery in below-ground and above-ground biomass. Bars = standard error.



*Figure 3.* Volatilized ammonia: total, coming from the soil and coming from the fertilizer. N soil: nitrogen volatilized from the soil; N fertilizer: nitrogen volatilized from fertilizer; N total: total nitrogen volatilized. Bars = standard error.

(1984) and Fapohunda and Hussain (1990). Only around 3% of the nitrogen from fertilizer was recovery in roots.

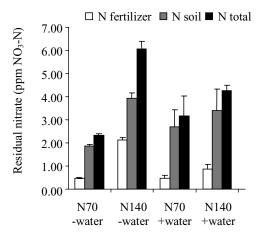
### Ammonia Volatilization

Total ammonia losses (NH<sub>3</sub>-N from fertilizer + NH<sub>3</sub>-N from soil) in greenhouse showed an interaction between N and water treatments (p = 0.016). Total ammonia volatilized significantly higher in +water/N140 treatment (Figure 3). The same was observed with the ammonia from fertilizer (Table 3; Figure 3). Losses of ammonia from soil were higher with the higher fertilization dose, independently of water regime (Table 3, Figure 3). Two possible explanations may account for this observation: i) the pH increases caused by high fertilization doses could increase the ammonia volatilization from the soil; ii) The isotopic exchange between  $^{15}$ N and  $^{14}$ N, by which the natural soil N ( $^{14}$ N) is overestimated (Harmsen and Moraghan, 1988). Natural ammonia volatilization (NH<sub>3</sub>-N from soil) is around 20% of total ammonia losses (NH<sub>3</sub>-N from soil + fertilizer).

Nitrogen losses via volatilization were 3.7 to 7.8% of N applied as fertilizer. A week after fertilization 64% of volatilized N came from the fertilizer. This proportion changed dramatically in the following weeks: at the end of the experiment only 20% of volatilized N came from such source (data not shown).

# **Residual Nitrate**

Nitrate measured at harvest showed significant differences among treatments, showing significant interactions between factors (Table 3). In agreement with



*Figure 4.* Residual nitrate at harvest time in the soil: total, coming from the soil and coming from the fertilizer. N soil: nitrate-nitrogen from the soil; N fertilizer: nitrate-nitrogen from fertilizer; N total; nitrate-nitrogen total. Bars = standard error.

the fertilizer recovery by plants, soil nitrate after harvest were higher in -water/N140 treatment (Figure 4). This nitrate concentration in the soil, either total N or  $^{15}$ N from fertilizer, confirmed the accumulation of nitrate in soils. A linear relationship was found between nitrate-N from the fertilizer in the soil at harvest time, and accumulated N from the fertilizer in total plant biomass ( $r^2 = 0.54$ ; p < 0.001) (equation 4).

$$NO_{3}$$
N fertilizer (ppm) = 2.322 - 0.0374 \* Fertilizer recovery (%) (4)

When the plants accumulated more N coming from the fertilizer, the residual soils nitrate were smaller. The lower N absorption due to water deficit and the higher N fertilization means a higher risk of nitrate leaching, and then groundwater contamination (Rimski-Korsakov et al., 2004).

## Fertilizer Fate

Nitrogen uptake was one of the main detected fates of the N applied (Fig ure 5). Plants recovered around 45% of the N applied, except in –water/N140 treatment where the recovery was as low as 19%. An important proportion of applied N was undetected in the studied compartments. Ammonia volatilization and residual nitrate were sinks for N applied of similar magnitude, with the exception of –water/N140 treatment. Residual nitrate were significantly higher in this treatment. There are other sinks for the applied N, but they do not appear to be important in these soils. Denitrification is a negligible process in the

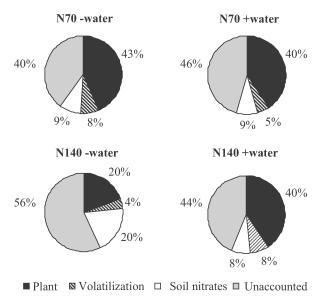


Figure 5. Fate of the fertilizer applied at greenhouse experiments.

agricultural soils of the Pampas as showed by Steinbach and Alvarez (2006). Non exchangeable ammonium was very stable in local soils to act as a sink for applied N, according to Rubio and Lavado (1994). Surface runoff can be discarded in containers. Main sink may be the incorporation of the applied N into the soil organic fraction. Portela et al. (2006) and Rimski-Korsakov (unpublished data) showed that in the Pampas around 30% of <sup>15</sup>N was found in the soil organic matter after harvest of maize.

#### CONCLUSIONS

Water deficit affected maize aerial and root biomass differently, and the observed results only partially agree with previous research. Among the differences between our results and those reported in the literature can be mentioned that maize subjected to water stress did not decrease root and aerial biomass production after nitrogen fertilization in present research. Also, N accumulation in aerial biomass was more affected by water stress than for N fertilization. Those differences can be accredited to differences in the level and characteristics of the water stress applied or suffered in the experiments.

With an adequate water regime the N recovery did not depend of the fertilizer doses. Water deficit decreased the N recovery by plants causing the parallel increase of residual nitrate. Despite other main destinations (N losses via volatilization, N incorporation into soil organic matter and N accumulation

in plant tissues) in maize fertilized and subjected to water stress, residual nitrate is an important destination. This fact can lead to the eventual nitrate leaching and its movement toward groundwater.

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